JOURNAL OF AGRICULTURAL RESEARCH

VOL. 78 WASHINGTON, D. C., MAY 15, 1949 No. 10

THE EFFECT OF EARTHWORMS ON THE PRODUCTIVITY OF AGRICULTURAL SOIL[1]

By HENRY HOPP, *soil conservationist*, and CLARENCE S. SLATER, *soil scientist*, *Soil Conservation Service, United States Department of Agriculture*

INTRODUCTION

Recent studies by the writers have brought out two points that bear on the relation of earthworms to agricultural soils: (1) Over widespread areas, the number of earthworms in crop fields is sufficient to influence markedly the structure of the soil (*10*, *26*),[2] and (2) the harmful effect of row cropping on the earthworm population can be overcome by establishing a protective cover on the ground by late fall (*14*). These findings lay a partial ground work for the development of practices that maintain earthworms in row-cropped land. However, the advantage to crops that may accrue from the presence of earthworms has not been adequately investigated.

It is recognized that earthworms have various chemical and physical effects on soil that should benefit productivity. They perforate the ground, thereby increasing aeration and infiltration (*3*, *8*, *9*, *17*, *18*, *20*, *24*, *28*, *29*). Their casts are relatively water stable, which should help in maintaining the structure intact under the dispersing action of rain water (*5*, *7*, *10*, *12*). They are effective chemically in that they mix organic surface debris with the inorganic fraction to form humus material, thereby concentrating the nutrients in parts of the soil where they are more available for crop growth (*2*, *3*, *4*, *9*, *17*, *18*, *19*, *21*, *22*, *23*, *24*, *25*, *28*, *29*).

Such observations of the effect of earthworms on soil have stimulated efforts to test the effect of earthworms on the yield of crops. Some of the first work along this line was reported in 1890 by Wollny (*30*), who found that when earthworms were added to soil in pots, marked increases in yield were obtained with a number of crops. Later workers (*1*, *6*, *17*, *21*, *24*) have repeated these experiments in one form or another. In general, the published results have confirmed Wollny's findings. When earthworms have been added to soil, either in pots or in the field, increases in yield have resulted.

Despite these favorable reports, agriculturists have hesitated to accept the results. This situation stems from at least four important and highly valid criticisms:

[1] Received for publication September 23, 1948. Cooperative investigation of Soil Conservation Service Research and the Maryland Agricultural Experiment Station. Published as Scientific Article No. A–211, Contribution No. 2131, of the Maryland Agricultural Experiment Station.

[2] Numbers in parentheses refer to Literature Cited, p. 338.

1. It has been reported in certain of the experiments that earthworms inoculated into the soil were found to have died by the end of the study. Since earthworms contain a high concentration of organic nitrogen, it has appeared that the beneficial effect of the inoculation on yield might be attributable simply to nitrogen fertilization. If the only effect is chemical, the same result could be obtained with chemical fertilizers and the earthworms would have no particular merit.

2. In others of the experiments, earthworm casts were collected and compared with the remaining soil in respect to the effect on crop yields. The criticism of this technique is that the casts contained much more of the organic matter and were therefore richer in the nutrients derived from plant residues. Agriculturists point to the well-known beneficial effect of incorporating organic matter into soil through cultural operations as evidence that the earthworms do nothing that cannot be done without them.

3. Other reports concern experiments in which soil was taken from areas that differed greatly in earthworm population and the productivity of these areas was compared under controlled conditions. The differences found in productivity have been ascribed by these investigators to the earthworms. Such experiments are justifiably subject to severe criticism in that they fail to demonstrate that the earthworms caused the good soil conditions. Agriculturists have been inclined to take the more straightforward viewpoint that the larger earthworm populations on better soils are due to more favorable conditions for their survival and reproduction.

4. The severe criticisms listed above are given added weight when it is considered that not all investigators have been able to obtain positive responses from inoculating earthworms under experimental conditions. It is true that the scientific reports are practically unanimous in showing positive effects. But negative results have been obtained, to the authors' knowledge. They are not reported in the literature probably because the investigations were abandoned at a preliminary stage.

The investigations reported in this paper were undertaken with a recognition of the criticisms to which previous work had been subject. Several tests were made under different conditions and variables were introduced that might help in clarifying the underlying causes of the responses shown by the crops. Owing to the diverse nature of the experiments, each will be described separately but they will be discussed and interpreted together at the end of the paper.

EXPERIMENTAL DATA

There are five experiments reported in this paper. In experiments 1 to 4 the species of earthworms used were a mixture of *Helodrilus caliginosus* forma *trapezoides* Duges and *Diplocardia* sp. Garman. In experiment 5 these species were used and in additoin *Lumbricus terrestris* L. and *Helodrilus chloroticus* Savigny. These are species commonly found in the vicinity of Beltsville, Md. The earthworms were stored for several days in the same soils as those used in the experiments in order to remove the previous contents of their alimentary tracts.

1. MILLET

Twelve glazed crocks were filled with a Christiana loam from which all earthworms had been removed. The soil was in good physical condition and had a moderately good organic content. Four sets were used as follows, with 3 replicates in each set:

Set 1. No worms added.
Set 2. 23 living worms placed in each of the crocks on May 10, 1946; these worms were removed on June 10, 1946.
Set 3. 23 dead worms placed in each of the crocks on May 10, 1946.
Set 4. 23 living worms placed in each of the crocks on May 10, 1946.

The worms were removed from the crocks of set 2 by spreading the soil on paper and examining it thoroughly. At the same time, all the other crocks were similarly handled. This treatment was somewhat similar to cultivation, since it left the soil in a crumbled condition.

On June 11, 1946, German millet was planted in the crocks at a rate equivalent to 50 pounds per acre. On July 8, the plants were thinned to 15 per crock. The millet was harvested on August 26, 1946.

During the period of the experiment, the crocks were placed in light shade outdoors and were watered occasionally.

The yield of millet in terms of oven-dry weight per stalk and the nitrate content of the soil on August 9, at which time the millet had ceased growth and was beginning to cure, is given in table 1. The

TABLE 1.—*Nitrate content of the soil and Millet yields at time of maturity for each crock*

Earthworm treatment	Oven-dry weight per millet stalk	Soil nitrate
	Grams	*P. p. m.*
1. None	2.3	4.1
	2.1	3.8
	1.8	3.2
	2.1	3.7
2. Living earthworms for 30 days prior to planting millet	1.8	4.5
	2.3	3.5
	2.2	6.4
	2.1	4.8
3. Dead earthworms	3.2	10.0
	2.2	7.5
	2.8	8.6
	2.7	8.7
4. Living earthworms	3.7	7.5
	2.6	7.5
	2.8	5.6
	3.0	6.9

number of stalks per crock varied slightly but without any relation to the treatments or to the weight per stalk.

The millet in the crocks that contained living worms throughout the period of the experiment had the highest average weight per stalk, 3.0 gm. However, the crocks with dead worms gave almost as high a value, 2.7 gm. The difference between the two sets was not significant. The crocks without worms or those from which the worms had been removed before the millet was planted showed much poorer growth, the average being 2.1 gm.

These differences in growth appeared to be associated in part with the nitrate content of the soil. The highest nitrate concentration was

in the crocks receiving dead worms, 8.7 p.p.m. In the crocks with living worms, the concentration was somewhat less, 6.9 p.p.m. Still, this value was almost twice that for the uninoculated crocks. The crocks from which the living worms had been removed prior to planting showed only slightly more nitrate than the check.

The high nitrate concentration in the crocks receiving dead worms had an obvious explanation; the nitrate was released in the decomposition of the worms' bodies. But the high nitrate concentration in the crocks containing live worms was not explainable until the soil was removed and examined for earthworms. It was found to contain an average of nine eggs and young worms per crock. Evidently the mature worms placed in the crocks had reproduced and died. Since such a reproductive phase is a normal occurrence in the life cycle of earthworms in the field, the phenomenon observed in the crocks had practical significance. This application will be considered in the discussion.

2. LIMA BEANS

The same 12 crocks were used for this test as for test No. 1. After the millet was harvested and the earthworms counted on August 26, 1946, the soil was put back in the crocks. The crocks were then placed outdoors in an unsheltered location. They were left fully exposed to the elements throughout the winter of 1946–47, and the following summer. On December 10, 1947, they were brought into the greenhouse and cultivated to a depth of about one-half inch. There was no evidence of living earthworms in the crocks. Calcium carbonate equivalent to 1 ton of limestone per acre was worked into the cultivated soil.

The 12 crocks were rearranged into 3 sets. The 4 crocks in each set consisted of 1 from each of the 4 variables of the millet experiment. In this way, any possible hold-over effect of the millet experiment on the growth of lima beans could be detected. However, no such effect was noted and this point will therefore be disregarded in the ensuing discussion. Apparently, the one and a half years the soil was exposed without earthworms to the elements served to overcome any differences that may have remained at the end of the millet experiment.

The three sets were treated as follows:

Set 1. No worms added.
Set 2. Ten dead earthworms placed in each of the crocks on December 10, 1947.
Set 3. Ten living earthworms placed in each of the crocks on December 10, 1947.

The greenhouse was maintained at natural temperature with artificial heating only when necessary to prevent freezing. The crocks were watered as necessary during the period of the experiment. There was considerable evidence of earthworm activity in the inoculated soil during the winter period. On April 22, 1948, the soil was fertilized with 4–8–12 at the rate of 1,000 pounds per acre, and one dwarf lima bean planted in each crock. The beans were harvested on August 2, 1948. The yields are shown in table 2.

The highest average yield was obtained in the set that had been inoculated with living earthworms. The set containing dead earthworms yielded almost but not quite as much. As in the millet ex-

TABLE 2.—*Yields of lima beans and pods in each crock*

Earthworm treatment	Air-dry weight of pods	Air-dry weight of beans
	Grams	*Grams*
1. None	7.3	5.0
	6.8	4.7
	0.8	0.0
	8.5	5.9
	5.8	3.9
2. Dead earthworms	23.4	14.8
	16.3	11.3
	5.6	3.3
	19.0	13.0
	16.0	10.6
3. Living earthworms	11.0	7.0
	15.5	10.3
	14.7	9.6
	26.3	19.2
	16.9	11.5

periment, the difference between these two sets was not significant. But the set without earthworms showed very much poorer growth. These differences are shown in figure 1. This experiment indicated

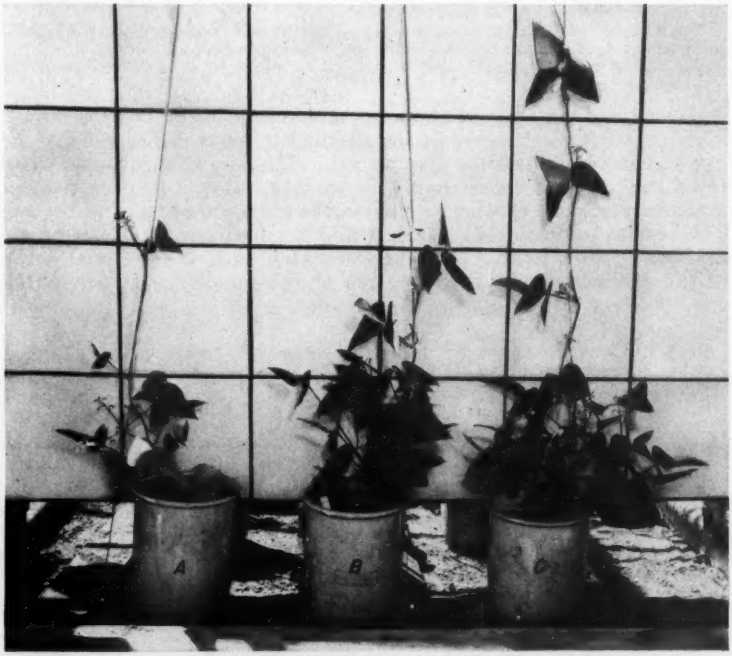

FIGURE 1.—Lima bean experiment: *A*, No earthworms added; *B*, dead earthworms added; *C*, living earthworms added. This soil was in good structure and showed a markedly stimulating effect from the chemicals present in the bodies of the earthworms but only a slight additional effect from the activity of the living earthworms.

that the chief effect of the earthworms could be attributed to chemicals in their bodies. But there was a suggestion of a slight additional effect from the action of living earthworms on the soil.

3. SOYBEANS

Ten wooden frames, measuring 8 inches long, 3 inches wide, and 6 inches high, and fitted with a glass front, were filled with the same type of soil as that used in the millet and lima-bean experiments. All worms were removed from the soil before it was placed in the frames. The soil was treated with 4–8–12 at a rate corresponding to 1,000 pounds per acre and hydrated lime at 2,000 pounds per acre. The test was started on June 3, 1947, at which time earthworm inoculations were made. The frames were stored in a cold-storage room at 55° F. until September 9, when they were placed under light shade in a greenhouse. One Black Wilson soybean was planted in each frame. The vegetation was harvested on November 26, 1947. Five sets were used with each set in duplicate, as follows:

Set 1. Soil maintained in its natural structure; no worms added.
Set 2. Soil puddled to destroy its natural structure; no worms added.
Set 3. Soil puddled, 10 dead worms added to each frame on September 9, 1947, the day the soybeans were planted.
Set 4. Soil puddled, 10 living worms added to each frame on June 13, 1947, approximately 3 months before the soybeans were planted.
Set 5. Soil puddled, dried, and broken into crumbs to simulate restoration of its original structure.

The oven-dry weight of above-ground vegetation is given in table 3. The soybeans grew poorly in the puddled soil and in the puddled soil in which dead earthworms were placed. They grew much better where the natural soil structure had been maintained or had been restored artificially, as well as where living earthworms were placed in the soil. The results indicate that the benefit from living earthworms on the soybeans grown in this soil was associated with the physical action of the earthworms on the structure of the puddled soil rather than the release of nutrients from their bodies.

TABLE 3.—*Soybean yields in each frame*

Soil and earthworm treatment	Oven-dry weight of tops
	Grams
1. Natural soil structure	4.20 1.71 2.96
2. Soil puddled	.56 -- .56
3. Soil puddled, dead earthworms added when soybeans were planted	.52 .79 .66
4. Soil puddled, living earthworms added	2.48 2.13 2.30
5. Soil puddled, structure artificially restored	2.30 3.82 3.06

4. WHEAT

The same 10 frames were used for this test as for test No. 3. After the soybeans were harvested on November 26, 1947, the frames were left for several days. Then, on December 5, they were fertilized with 4–8–12 at the rate of 500 pounds per acre and fresh cow manure at the rate of 10 tons per acre. These materials were worked into the top one-half inch of soil. The frames of set 4 were reinoculated with 10 living worms each. On December 15, 10 dead worms were worked into the surface soil in the frames of set 3. The next day, 3 wheat grains were planted in each frame. During the winter, the plants were kept in a greenhouse at natural temperature with artificial heating only when necessary to prevent freezing. The wheat grew slowly during the winter, but in the spring became active again. Differences in growth began to appear among the sets and it became evident that the better growth was taking place in the frames that had been inoculated with living earthworms. In order to preclude the effects of the ordinary nutrients, all frames were refertilized on March 11, 1948, with 10–6–4 in liquid form at the rate of 300 pounds per acre, and on March 19, with fresh manure at the rate of 10 tons per acre. The wheat matured normally and was harvested on June 16, 1948. The yields are given in table 4. The number of wheat grains parallel the weight of the stalks fairly well.

The puddled soil (set 2) showed distinctly poorer growth despite the heavy fertilization and manuring. The addition of dead earthworms increased the yield, but the highest average yield was obtained with the living earthworms. These data indicate that the wheat benefited from chemicals in the earthworms' bodies other than those in the fertilizer and the fresh manure that had been applied, as well as from the effect of the living earthworms on the soil.

TABLE 4.—*Wheat yields in each frame*

Soil and earthworm treatment	Air-dry weight of stalks		Wheat grains	
	Grams		*Number*	
1. Natural soil structure	9.4		109	
	9.7		132	
		9.5		120
2. Soil puddled	8.7		80	
	5.5		41	
		7.1		60
3. Soil puddled, dead earthworms added when wheat was planted	8.1		82	
	10.0		103	
		9.0		92
4. Soil puddled, living earthworms added	9.4		131	
	11.5		88	
		10.5		110
5. Soil puddled, structure artificially restored	7.7		94	
	10.9		80	
		9.3		87

5. MIXED HAY

The experiment with mixed hay was conducted on an earthworm-free clay subsoil in barrels that measured 32½ inches in height and 20 inches top diameter (fig. 2). The barrels were placed outdoors

FIGURE 2.—Hay experiment: *A*, Dead earthworms added; *B*, living earthworms added. Suitable cover conditions were maintained in both barrels for the winter survival of earthworms. The soil was in poor structure and showed a large stimulating effect on clover from the addition of living earthworms.

throughout the period of the experiment so that their climatic environment was fairly natural. The data reported in this paper are only a part of the experiment, the remainder is not pertinent. The procedure will be described only briefly since details have been given in a previous article (*16*).

In the fall of 1946, the soil in the barrels was liberally limed, manured, fertilized, and seeded to a mixture of grasses and legumes. Despite the treatments, the barrels supported only a moderate growth of weedy vegetation in 1947 because of the original unproductivity of the soil. In the fall of 1947, living earthworms were placed in the soil of 10 out of the 20 barrels that are described in this article. The introduction was equivalent to a population of 339,000 per acre. The other 10 barrels, which served as checks, were inoculated with the same number of dead earthworms. In April 1948, after the soil had thawed, the barrels were again inoculated with earthworms.

The barrels were treated by pairs, one of each pair having received living earthworms, the other not. The treatments applied to the pairs were as follows:

Set 1. Sod clipped in the fall of 1947, clippings left on surface.

Set 2. Same as set 1, except in addition, top-dressed with 300 pounds per acre of 4–8–12 in the fall of 1947 and again in the spring of 1948.

Set 3. Sod clipped in the fall of 1947, clippings removed, and soil surface either covered with asphalt roofing paper or left bare.

Set 4. Same as set 3, except in addition, top-dressed with 300 pounds per acre of 4–8–12 in the fall of 1947 and again in the spring of 1948.

The hay was harvested from the barrels in June of 1948. Yields are shown in table 5.

In sets 1 and 2, where the clippings were left on the surface in the fall so as to keep the earthworms alive over the winter (*11*, *14*), the barrels inoculated with living earthworms bore almost four times as much vegetation as the barrels inoculated with dead earthworms (fig. 2). The effect of the living earthworms was somewhat greater in the barrels receiving a top dressing of fertilizer in the spring. In sets 3 and 4, where the soil surface was left either bare or with only a light asphalt paper covering over winter, inoculation with living earthworms had much less effect on yield.

TABLE 5.—*Hay yields in each barrel*

Overwinter cover	Top-dressed in spring	Air-dry weight of vegetation		
		Inoculated with living earthworms	Not inoculated with living earthworms	Apparent effect of inoculation
		Grams	*Grams*	*Grams*
Sod clippings	No	64.3 67.0 65.6	13.1 16.1 14.6	51.0
Do.	Yes	103.7 74.7 89.2	27.8 26.9 27.4	61.8
None or asphalt paper	No	25.0 26.2 58.3 36.5	7.6 9.8 15.6 11.0	25.5
Do.	Yes	21.5 28.8 60.8 37.0	19.0 23.2 [1] 55.0 32.4	4.6

[1] This barrel infested with ants.

The beneficial effect of the living earthworms in the barrels with suitable cover conditions seemed to be due to their physical action on the structure of the soil. An ant infestation, which occurred in the early part of 1948 in one of the barrels, had a similar stimulating effect. It appears, however, that under outdoor conditions this physical action of earthworms is effective primarily during the winter season; for the reinoculation, made in the spring of 1948, did not overcome the loss of the earthworms in the barrels that had insufficient late fall protection.

The stimulation of the vegetation under the action of the earthworms was confined almost entirely to the growth of clover. Where living earthworms were not introduced or where they were added but unsuitable cover conditions established for their survival, the vegetation was chiefly grass, weeds, and later in the summer, lespedeza. Where the earthworm population was favored, the growth was largely clover with only a small proportion of grass, weeds, and lespedeza. The infestation by ants likewise stimulated the clover.

836173—49——2

DISCUSSION

The results of these various experiments are brought together in table 6. Since the same set of variables was not used in every experiment, only the comparisons between no earthworms, dead earthworms, and living earthworms are given in the summary table.

The addition of dead earthworms increased the yields by an average of 81 percent. The trend was consistent for all the experiments, although the actual magnitude of the increase was small in the soybean experiment.

TABLE 6.—*Summary of the experiments*

Experiment	Yields of vegetation			Benefit of—	
	No earthworms	Dead earthworms	Living earthworms	Dead earthworms over no earthworms	Living earthworms over dead earthworms
				Percent	*Percent*
1. Millet: Weight per stalk.....grams..	2.1	2.7	3.0	29	11
2. Lima beans:					
Beans.....grams..	3.9	10.6	11.5	175	8
Pods.....grams..	5.8	16.0	16.9	183	6
3. Soybeans: Weight of tops.....grams..	.56	.66	2.30	18	248
4. Wheat:					
Weight of stalks.....grams..	7.1	9.0	10.5	27	17
Number of grains.....	60	92	110	53	20
5. Hay (sod clippings over winter): Weight of vegetation.....grams..	.....	21.0	77.4	.....	269
Mean.....	.....	.....	.....	[1] 81	[2] 83

[1] Average increase in yield due to dead earthworms.
[2] Additional increase in yield due to living earthworms over the increase due to dead earthworms.

However, the chemicals added in the bodies of the earthworms were not the entire explanation of the effects of earthworms on yield. Although the dead earthworms consistently increased yields over no earthworms, the living earthworms had a marked additional effect. The yield with living earthworms was 83 percent greater than with dead earthworms. This effect was consistently positive in all experiments. It was especially marked in the soybean and hay experiments, and rather slight in the other experiments.

The variation in the effect of living earthworms as compared with dead earthworms was explainable by crop and soil differences. If, for the moment, we disregard the hay experiment, we may consider the other four experiments as a unit, for all were conducted on soil from a single field. Originally, this soil had good structure and contained a large earthworm population. In the millet and lima-bean experiment, the soil was placed in the containers without destroying its aggregate structure. In the soybean and wheat experiments, the soil was puddled in order to destroy its structure.

The soybeans and wheat responded differently to the loss of structure. Puddling reduced the yield of soybeans 81 percent (from 2.96 to 0.56 gm., as shown in table 3). But the yield of wheat was reduced by puddling only 25 percent (from 9.5 gm. to 7.1 gm., as shown in table 4). The addition of dead earthworms to the puddled soil bene-

fited the soybeans only slightly, but the wheat markedly. Evidently soil structure was limiting the growth of soybeans to such an extent that they could not respond to the chemicals provided in the earthworms' bodies. With the wheat, where structure was having only a slightly depressive effect, the plants were able to respond to the addition of the chemicals.

The difference among crops in their tolerance to poor soil structure, as exemplified herein by soybeans and wheat, is evidently a factor that must be considered in evaluating the effect of earthworms on yield.

The millet and lima beans, growing in soil of good structure, responded markedly to the addition of dead earthworms. Here the structure was not limiting and the chemicals added through the bodies of the earthworms had a stimulating effect on growth. The living earthworms increased the yield only slightly over the dead earthworms, and the difference in favor of living earthworms was not significant with either crop. Yet it was consistent in both and therefore probably real. There was evidently a small additional benefit from the activity of living worms.

The hay experiment differed from the four others as regards the soil used. It was an extremely unproductive clay subsoil of very poor structure. The growth of hay was poor despite the cultivation given this soil in the original preparation of the seedbed; the very liberal use of lime, fertilizer, and fresh manure; and the addition of dead earthworms. The factor retarding growth was evidently not the lack of chemicals. But when living earthworms were introduced with favorable cover for their survival, vegetation was greatly stimulated. The beneficial effect was associated with improved structure as indicated by infiltration tests as well as by a similar effect from the infestation of ants that likewise loosened the soil. The response was mainly in the growth of clover rather than in the grass, weeds, or lespedeza. This experiment, then, gave further evidence that the difference among crops in their tolerance to poor soil structure is a factor in the effect of earthworms on yield.

Since both living and dead earthworms had stimulating effects according to the limiting factors of the soil, consideration should be given to the manner in which these effects may operate in the field.

In a previous study (*11*) it has been shown that earthworms follow a life-cycle pattern related to ecological factors of the soil. In order to clarify these relationships, one of the illustrations of the earlier study is reproduced herein (fig. 3). As shown in the illustration, earthworms reach maturity during the winter and spring months and then reproduce. The parent generation largely dies during the summer and is replaced by a new generation. On cultivated land that has insufficient protection in the late fall to maintain the earthworms, they die in early winter.

The present study suggests that the maintenance of the normal life cycle, characterized by death of the mature earthworms during the summer, may be important to the growth of crops. The beneficial chemicals in the dead earthworms would then be released at a time when crops are able to use them. On the other hand, the upset in this normal cycle, when late-fall protection is insufficient, results in

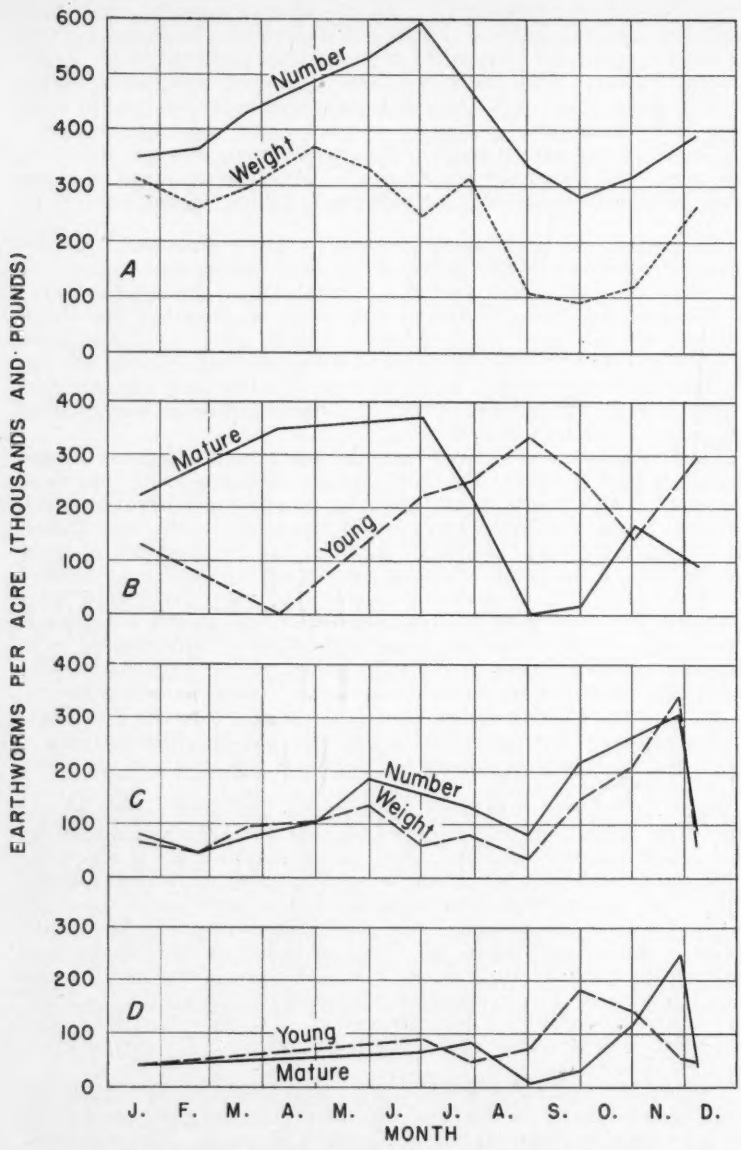

FIGURE 3.—The annual earthworm cycle in a field at College Park, Md. On sod ground (*A* and *B*), the mature earthworms reproduced and died during the summer; on cultivated ground that had insufficient protection in the late fall (*C* and *D*), the earthworms for the most part died in the early part of the winter.

the release of these chemicals at a time when crops are dormant and unable to use them.

Identification of the stimulating chemicals requires further investigation. As shown in the millet experiment, the death of the earthworms released a noticeable amount of nitrate; and nitrate was probably the most critical nutrient in this soil. The release of nitrate and other ordinary essential elements from the earthworms' bodies may account in part for their beneficial chemical effect. But in all the experiments, liberal quantities of commercial fertilizer were used and yet the dead earthworms increased yield. In the wheat experiment, fertilizer was applied in the fall and again in the spring, the total application being equivalent to 50 pounds of N, 58 pounds of P_2O_5, and 72 pounds of K_2O per acre. In addition, 10 tons per acre of fresh cow manure was worked into the soil in the fall, and 10 tons more applied as top dressing in the spring. Still the dead earthworms increased yield. It therefore appeared that the beneficial chemicals in the bodies of earthworms, whatever their nature, produced effects that were not obtained from the commercial fertilizer or the fresh cow manure used.

Also the beneficial physical activity of living earthworms is dependent on the maintenance of their normal life cycle. In the present study, it was found that the activity of living earthworms was especially effective in soil with poor structure. The poor structure was attained in experiments 3 and 4 by artificially puddling a well-aggregated soil, and in experiment 5 by selecting a clay subsoil that was of extremely poor structure. In this respect, therefore, the soils were not typical of most soils used for agriculture. However, it is widely recognized that the loss of soil structure often does become a limiting factor under field conditions. In two papers (*16*, *27*) published in conjunction with this paper, it is shown that much loss of structure is associated with the winter season and that the maintenance of the earthworm population during the winter is associated with benefits to soil structure. This physical activity was found in the hay experiment of the present paper (experiment 5) to be effective with earthworms introduced in the fall and not with those introduced in the spring. While earthworms can affect soil structure at almost any time of the year, if suitable moisture and temperature conditions are provided, the evidence from these experiments and field observation indicates that under actual farming conditions their physical activity in soil occurs largely from fall to spring. During the summer, when the soil becomes warm and dry, earthworms have much less influence on the structure of the soil. The benefits of earthworms on soil structure are therefore dependent on maintaining the population over winter.

SUMMARY

Tests were made to determine the effect of earthworms on the productivity of soil.

In five tests, with different crops and soils, earthworms consistently increased yields. Their influences varied widely according to crop and soil.

The increases in yield were attributable to the release of beneficial

chemicals from the bodies of the earthworms and to their physical effects on soil structure.

The beneficial chemicals were not identified, but their effects were not offset by liberal application of N–P–K fertilizer and fresh cow manure.

Available evidence suggests that the release of beneficial chemicals occurs principally in the summer season when earthworms normally pass through their reproductive period.

Beneficial physical effects on soil structure were obtained on soils where the lack of structure was limiting to crop growth.

The crops differed in their tolerance to poor soil structure and therefore in their response to the activity of living earthworms. Soybeans and clover were stimulated more than wheat and grass by the activity of living earthworms in soil of poor structure.

Benefits from the physical activity of living earthworms were obtained at various seasons depending on favorable soil moisture and temperature, but available evidence indicates that under the field conditions examined, this activity is largely effective during the late fall, winter, and early spring.

In one of the experiments, with soil of poor structure, ants produced beneficial effects on yield similar to those of earthworms; an observation which suggests that soil fauna other than earthworms may also have far-reaching importance to the productivity of soils.

LITERATURE CITED

(1) Archangelski, M.
1929. [On the influence of earthworm activity on yields of oats and barley in relation to the application of various fertilizers.] Jour. Agr. Sci. [Russia] 6: 849.

(2) Bates, G. H.
1933. The distribution of wild white clover (Trifolium repens) in relation to the activity of earthworms (Lumbricidae). Welsh Jour. Agr. 9: 195–208.

(3) Blanck, E., and Giesecke, F.
1924. Ueber den einfluss der regenwürmer auf die physikalischen und biologischen eigenschaften des bodens. Ztschr. f. Pflanzenernähr., Düngung u. Bodenk. 3 (B): 198–210.

(4) Darwin, C.
1882. The formation of vegetable mould through the action of worms. 326 pp., illus. New York.

(5) Dawson, R. C.
1948. Earthworm microbiology and the formation of water-stable soil aggregates. Soil Sci. Soc. Amer. Proc. (1947) 12: 512–516.

(6) Dreidax, L.
1931. Untersuchungen über die bedeutung der regenwürmer für den pflanzenbau. Arch. f. Pflanzenbau 7: 413–467.

(7) Dutt, A. K.
1948. Earthworms and soil aggregation. Amer. Soc. Agron. Jour. 40: 407–410.

(8) Evans, A. C.
1948. Studies on the relationships between earthworms and soil fertility. II. Some effects of earthworms on soil structure. Ann. Appl. Biol. 35: 1–13.

(9) Hensen, V.
1882. Ueber die fruchtbarkeit des erdbodens in ihrer abhängigkeit von den leistungen der in der erdrinde lebenden würmer. Landw. Jahrb. 11: 661–698.

(10) Hopp, H.
1946. Earthworms fight erosion, too. Soil Conserv. 11: 252–254.

(11) Hopp, H.
1948. THE ECOLOGY OF EARTHWORMS IN CROPLAND. Soil Sci. Soc. Amer. Proc. (1947) 12: 503–507.
(12) ———, and Hopkins, H. T.
1946. EARTHWORMS AS A FACTOR IN THE FORMATION OF WATER-STABLE SOIL AGGREGATES. Jour. Soil and Water Conserv. 1: 11–13.
(13) ———, and Hopkins, H. T.
1946. THE EFFECT OF CROPPING SYSTEMS ON THE WINTER POPULATION OF EARTHWORMS. Jour. Soil and Water Conserv. 1: 85–88, 98.
(14) ———, and Linder, P. J.
1947. A PRINCIPLE FOR MAINTAINING EARTHWORMS IN FARM SOILS. Science 105: 663.
(15) ———, and Slater, C. S.
1948. INFLUENCE OF EARTHWORMS ON SOIL PRODUCTIVITY. Soil Sci. 66: 421–428.
(16) ———, and Slater, C. S.
1948. A PRINCIPLE FOR MAINTAINING STRUCTURE IN CLEAN-CULTIVATED SOILS.
(17) Kahsnitz, H. G.
1922. UNTERSUCHUNGEN ÜBER DEN EINFLUSS DER REGENWÜRMER AUF BODEN UND PFLANZE. Bot. Arch. I: 315–331.
(18) Lindquist, B.
1941. [INVESTIGATIONS OF THE IMPORTANCE OF SOME SCANDINAVIAN EARTHWORMS FOR THE DECOMPOSITION OF BROADLEAF LITTER AND FOR THE STRUCTURE OF MULL.] Svenska Skogsvårdsför. Tidskr. 39: 179–242.
(19) Lunt. H. A., and Jacobson, H. G. M.
1944. THE CHEMICAL COMPOSITION OF EARTHWORM CASTS. Soil Sci. 49: 367–375.
(20) Müller, P. E.
1887. STUDIEN ÜBER DIE NATÜRLICHEN HUMUSFORMEN, UND DEREN EINWIRKUNG AUF VEGETATION UND BODEN. 324 pp., illus. Berlin.
(21) Powers, W. L., and Bollen, W. B.
1935. THE CHEMICAL AND BIOLOGICAL NATURE OF CERTAIN FOREST SOILS. Soil Sci. 40: 321–329.
(22) Puh, Y. C.
1941. BENEFICIAL INFLUENCE OF EARTHWORMS ON SOME CHEMICAL PROPERTIES OF THE SOIL. Sci. Soc, China, Biol. Lab. Contrib., Zool. Ser. 15: 147–155.
(23) Robertson, J. D.
1936. THE FUNCTION OF THE CALCIFEROUS GLANDS OF EARTHWORMS. Jour. Expt. Biol. 13: 279–297.
(24) Russell, E. J.
1909. THE EFFECT OF EARTHWORMS ON SOIL PRODUCTIVENESS. Jour. Agr. Sci. [England] 3 (II): 246–257.
(25) Salisbury, E. J.
1924. THE INFLUENCE OF EARTHWORMS ON SOIL REACTION AND THE STRATIFICATION OF UNDISTURBED SOILS. Linn. Soc. London, Jour., Bot. 46: 415–425.
(26) Slater, C. S., and Hopp, H.
1948. RELATION OF FALL PROTECTION TO EARTHWORM POPULATIONS AND SOIL PHYSICAL CONDITIONS. Soil Sci. Soc. Amer. Proc. (1947) 12: 508–511.
(27) ———, and Hopp, H.
1949. THE ACTION OF FROST ON THE WATER-STABILITY OF SOILS. J. Agr. Res. 78
(28) Stöckli, A.
1928. STUDIEN ÜBER DEN EINFLUSS DES REGENWURMES AUF DIE BESCHAFFENHEIT DES BODENS. Landw. Jahrb. der Schweiz. 42: 5–121.
(29) Wollny, E.
1890. UNTERSUCHUNGEN ÜBER DIE BEEINFLUSSUNG DER FRUCHTBARKEIT DER ACKERKRUME DURCH DIE TÄTIGKEIT DER REGENWÜRMER. Forsch. auf dem Geb. der Agrikulturphys. 13: 381–395.

THE ACTION OF FROST ON THE WATER-STABILITY OF SOILS[1]

By CLARENCE S. SLATER, *soil scientist*, and HENRY HOPP, *soil conservationist*, *Soil Conservation Service, United States Department of Agriculture*

INTRODUCTION

Soil that had some form of insulative protection throughout the winter months was found recently to be in better physical condition the following spring than soil that lay bare over winter (*9*).[2] Infiltration rates and water-stability were higher for the protected soil. These benefits were associated in part with increased earthworm activity (*4*, *6*). On the unprotected soil, there was evidence that the structure had deteriorated during the winter months. The deterioration appeared to be related to the action of frost on the unprotected soil.

Few studies have been made of the effect of frost on soil. Baver (*1*) states that the effect of freezing and thawing is to granulate clods in the soil but adds that it does not always result in a highly aggregate soil. He cites investigations by Jung (*7*) in which soil moisture content and rate of freezing were found to modify the action of frost. The data appear to involve the action of frost on the flocculation of soil colloids. However, it is not clear that the data have any relation to the formation of soil aggregates. Likewise the studies by Gardner (*5*) concern the action of frost in flocculating dispersed colloids.

These previous works show that freezing and thawing affect soil, but whether the effects are beneficial or detrimental to aggregate structure is not clear. In view of the field observations made by the writers and the uncertainty in the literature, it was considered desirable to make a specific study of the effect of frost on soil structure.

EXPERIMENTAL PROCEDURE

The soils used for the study were obtained from experimental plots at Wooster and Holgate, Ohio; and at College Park, Md. The soil from Holgate was a clay loam of lacustrine origin, that from Wooster was a glacially derived noncalcareous silt loam. The soil from College Park was also a silt loam, but derived from coastal plains deposits. At each location two samples of soil were selected. They were of the

[1] Received for publication September 23, 1948. Cooperative investigation of Soil Conservation Service Research, and the Maryland Agricultural Experiment Station. Published as Scientific Article No. A212, Contribution No. 2132, of the Maryland Agricultural Experiment Station.

[2] Numbers in parentheses refer to Literature Cited, p. 346.

same soil type, but differed in physical quality as shown by water-stability tests. The differences in quality were the result of previous cropping practices.

Samples of dry soil weighing 100 gm. were placed in covered metal cans. Water was added to the samples slowly in measured amounts. Three different moisture levels were tested. The lowest level was equal to the moisture equivalent. The others were equal to 15 and 30 percent of moisture in excess of the moisture equivalent. After the water had distributed itself uniformly there appeared to be no free water in any of the samples.

The samples are divided into six treatment groups as follows:

1. Frozen and thawed rapidly one time.
2. Frozen and thawed slowly one time.
3. Frozen and thawed rapidly five times.
4. Frozen and thawed slowly five times.
5. Moistened and dried immediately.
6. Stored moist at 50° F. for the duration of the freezing tests.

Freezing was accomplished by placing the cans in a cold storage room at a temperature of 0° F. The slow rate of freezing was obtained by wrapping the cans in cotton batting and placing them in cardboard containers. The soil froze in about 5 hours. Samples for fast freezing were not insulated. They froze in about 1 hour.

Freezing caused an expulsion of considerable free water from the wettest samples. When the freezing tests were complete, the excess of free water was pipetted off and measured.

After this treatment, the frozen and unfrozen samples were allowed to air-dry at room temperatures and were crushed to pass a 5-mm. screen. The 5-3 mm. fractions were analyzed to determine water-stability (*3*). Water-stability is used in this study to show the effects of freezing on soil structure.

RESULTS

The soils frozen at high and at medium moisture content showed obvious evidence of physical damage. They were in a puddled condition and markedly slumped. Free water had been expelled by freezing (*2*). In the wettest samples it completely covered the surface of soil. In 26 out of the 48 samples, frozen at high moisture content, the free water amounted to more than 10 percent of the total that had been added.

The puddled condition of the samples as a result of freezing is reflected in the water-stability data which are given in table 1. The data were analyzed statistically, and effects of importance are presented in smaller summary tables.

TABLE 1.—*The water-stability of soils as affected by treatments*

Treatment	Soil from—					
	Holgate		Wooster		College Park	
	Poor quality	Good quality	Poor quality	Good quality	Poor quality	Good quality
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Low moisture:						
Dried immediately	48.0, 41.8	76.7, 76.8	31.3, 26.7	65.5, 68.0	39.3, 34.4	49.5, 56.2
Stored moist	54.0, 51.2	78.0, 74.1	29.9, 31.5	75.2, 69.6	35.3, 38.1	60.4, 63.5
Frozen rapidly, once	42.4, 46.3	73.8, 74.0	13.9, 20.1	58.1, 65.8	30.2, 34.6	58.0, 64.1
Frozen rapidly, 5 times	36.1, 37.6	63.2, 62.0	16.0, 19.9	61.0, 59.8	32.7, 30.4	55.8, 58.9
Frozen slowly, once	44.0, 43.2	73.6, 76.5	19.9, 21.8	59.1, 65.0	29.8, 34.5	58.2, 51.8
Frozen slowly, 5 times	41.8, 36.0	75.0, 71.8	17.7, 19.1	57.7, 66.5	33.7, 32.2	60.8, 58.2
Medium moisture:						
Dried immediately	38.9, 34.8	70.8, 71.2	25.3, 19.1	61.6, 59.3	25.5, 24.5	53.7, 46.1
Stored moist	38.4, 43.4	71.5, 74.0	24.5, 23.1	57.3, 64.1	28.8, 29.4	56.5, 56.6
Frozen rapidly, once	27.9, 28.4	59.2, 58.5	10.3, 11.7	55.0, 54.3	20.8, 22.6	46.5, 42.1
Frozen rapidly, 5 times	16.1, 22.8	39.2, 34.1	10.9, 12.6	46.7, 48.7	16.4, 14.4	28.5, 34.9
Frozen slowly, once	27.1, 28.8	55.4, 55.1	14.4, 15.7	50.4, 61.8	17.2, 19.5	31.1, 34.8
Frozen slowly, 5 times	19.2, 22.6	41.5, 40.2	11.6, 14.5	40.7, 45.8	20.7, 16.6	29.8, 33.9
High moisture:						
Dried immediately	30.9, 27.3	60.7, 64.2	17.6, 16.4	55.6, 61.1	19.7, 18.9	40.7, 44.0
Stored moist	29.5, 34.6	66.7, 69.9	16.5, 17.9	58.6, 63.3	22.4, 19.3	49.6, 43.2
Frozen rapidly, once	16.0, 16.6	39.0, 39.3	6.1, 8.5	32.8, 42.4	14.7, 13.2	25.6, 26.7
Frozen rapidly, 5 times	8.5, 9.7	23.5, 26.8	7.1, 8.0	28.5, 31.5	11.9, 9.2	15.5, 18.5
Frozen slowly, once	14.3, 17.5	46.1, 40.0	8.9, 8.2	39.1, 44.2	14.6, 13.9	18.6, 24.3
Frozen slowly, 5 times	10.5, 10.0	21.2, 26.3	6.9, 7.3	29.7, 23.8	10.8, 10.2	16.4, 20.1

Data on the effect of storing or freezing moist soil are summarized in table 2. The frozen soils suffered an average decrease in water-

TABLE 2.—*The effect of storing and freezing moist soils*

Soil treatment	Water-stability	Effect of treatment
	Percent	*Percent*
Moistened and dried immediately	45	
Stored moist	48	[1] 3
Frozen moist	33	[1] −12

[1] Significant beyond the 1-percent level.

stability of 12 percent, for all conditions of freezing. This decrease was attributable directly to the freezing and not merely to the moistening of the soil, for the soil that was stored moist actually increased in water-stability by an average of 3 percent. The decrease due to freezing and the increase due to moist storage were both highly significant statistically.

The effect of freezing on different types and qualities of soil is shown in table 3. All soils showed significant reductions in water stability as

TABLE 3.—*The effect of freezing soils of different types and qualities*

Soil type	Soil quality	Water-stability		
		Dried immediately	Frozen	Effect of freezing [1]
		Percent	*Percent*	*Percent*
Holgate	Poor	37	26	−11
	Good	70	51	−19
Wooster	Poor	23	13	−10
	Good	62	49	−13
College Park	Poor	27	21	−6
	Good	48	38	−10

[1] All effects significant beyond the 1-percent level.

a result of freezing. Soils of good quality suffered a larger reduction in water-stability than soils of poor quality. The better quality soils had a higher water-stability to begin with, hence there was more material to be disintegrated by freezing. However, the water-stability after disintegration by freezing remained higher in the better soils.

The moisture content at which the soils were frozen had a marked effect on the reduction in water-stability. Results are summarized in

TABLE 4.—*The effect of freezing at different moisture contents*

Moisture content above moisture equivalent	Water-stability		
	Dried immediately	Frozen	Effect of freezing [1]
Percent	*Percent*	*Percent*	*Percent*
0	51	47	−4
15	44	31	−13
30	38	20	−18

[1] All effects significant beyond the 1-percent level.

table 4. Freezing at the moisture equivalent caused only a small decrease in water-stability, whereas the greatest loss in water-stability was incurred at the highest moisture content.

Rate of freezing and thawing (table 5) had no significant effect on water-stability under the conditions of this experiment.

TABLE 5.—*The effect of slow and fast rates of freezing*

Moisture content above moisture equivalent	Water-stability		
	Frozen slow	Frozen fast	Effect of fast freezing [1]
Percent	*Percent*	*Percent*	*Percent*
0	48	46	2
15	31	32	−1
30	20	20	0

[1] All effects are nonsignificant.

Freezing and thawing repeatedly (table 6) decreased water-stability

TABLE 6.—*The effect of number of times of freezing*

Moisture content above moisture equivalent	Water-stability		
	Frozen once	Frozen 5 times	Effect of repeated freezing
Percent	*Percent*	*Percent*	*Percent*
0	48	46	−2
15	35	28	[1]−7
30	24	16	[1]−8

[1] Significant beyond the 1-percent level.

to a greater extent than freezing and thawing once. The difference was not significant at the lowest moisture content, but was highly significant at the higher moisture contents.

The above data show that the most drastic treatment was to freeze the soil repeatedly at a high moisture content. Soils subjected to this treatment showed very little remaining water-stability; they were almost completely dispersed. The data show that some water stability is left, but actually the material remaining on the sieves was mostly discrete sand particles. These were not distinguished from aggregated particles in the method of analysis that was used (*3*).

DISCUSSION

The damaging effect of frost on the water-stability of soils undoubtedly occurs frequently under field conditions. The puddled appearance and slumped condition of frozen soil as observed in this investigation can be seen in many fields in early spring, particularly those that lay bare over winter. According to the results of this study, one would expect to find the greatest damage where soils are frozen and thawed repeatedly, and where their moisture content is high.

In this connection the work of Post and Dreibelbis (*8*) is of interest. They found that a concentration of water at or near the soil surface is a characteristic of frozen soils, and that upon thawing the moisture content at the surface tends to approach saturation. It follows that to prevent increasingly greater damage by repeated freezing such excess water must be drained from the soil surface.

This study on the factors that influence the effect of frost on soils, and the writers' previous investigations (*6*, *9*), suggest a means of moderating frost damage. Insulative protection of mulch or vegetation reduces the number of times a soil freezes, and the depth to which frost penetrates. It promotes and maintains large populations of earthworms. These increase infiltration, thereby allowing the drainage of excess water out of the surface soil. By favoring the activity of earthworms the hazard of freezing at high moisture contents is reduced.

The uniformity with which frost damaged the water-stability in this study leads the writers to question whether frost ever promotes good structure in agricultural soils. It is true that the physical condition of protected soils often improves over winter. This may erroneously be attributed to frost action. Actually, improvement is due to other favorable factors, such as the protection from freezing and the stimulation of earthworm activity.

The writers in their experience have yet to find any structural improvement in fields that lay bare over winter. It has been noted that when clay soils are plowed in the fall, they are frequently friable and easily tilled in the spring. The frost action fractures the intractable lumps and clods. By the same token, bonds within the aggregates are likewise destroyed. The result is that the soil, while apparently in good condition because of its freedom from clods, actually has so little water-stability remaining that the favorable tilth produced by land preparation is not retained throughout the growing season. For this reason, attempts to maintain good structure in heavy soils by fall plowing have notably failed.

SUMMARY

Freezing and thawing decreased the water-stability of moist soils. The detrimental effect of freezing and thawing was most marked at high moisture contents.

The loss of water-stability was increased by repeated freezing and thawing.

Rate of freezing and thawing had no effect on the loss of water-stability.

Methods designed to overcome the harmful effects of frost action should be based on reducing the depth and frequency of freezing, and on maintaining drainage channels in the soil layer where freezing takes place in order to keep the moisture content as low as possible.

LITERATURE CITED

(1) Baver, L. D.
1948. soil physics. Ed. 2, 398 pp., illus. New York.

(2) Bouyoucos, G. J.
1921. the concentration of the soil solution around the soil particles. Soil Sci. 11: 131–138.

(3) Bryant, J. C., Bendixen, T. W., and Slater, C. S.
1948. measurement of the water-stability of soils. Soil Sci. 65: 341–345.

(4) Dawson, R. C.
1948. earthworm microbiology and the formation of water-stable aggregates. Soil Sci. Soc. Amer. Proc. (1947) 12: 512–516.

(5) Gardner, R.
1945. some effects of freezing and thawing on the aggregation and permeability of dispersed soils. Soil Sci. 60: 437–443.

(6) Hopp, H.
1948. the ecology of earthworms on cropland. Soil Sci. Soc. Amer. Proc. (1947) 12: 503–507.

(7) Jung, E.
1931. untersuchungen über die einwirkung des frostes auf den erdboden. Kolloid Chem. Beihefte. 32: 320–373.

(8) Post, F. H., and Dreibelbis, F. R.
1943. some influences of frost penetration and micro-climate on the water relationships of woodland, pasture, and cultivated soils. Soil Sci. Soc. Amer. Proc. (1942) 7: 95–104.

(9) Slater, C. S., and Hopp, H.
1948. relation of fall protection to earthworm populations and soil physical conditions. Soil Sci. Soc. Amer. Proc. (1947) 12: 508–511.

A PRINCIPLE FOR MAINTAINING STRUCTURE IN CLEAN-CULTIVATED SOILS[1]

By HENRY HOPP, *soil conservationist*, and CLARENCE S. SLATER, *soil scientist*, *Soil Conservation Service, United States Department of Agriculture*

INTRODUCTION

The deterioration in structure that occurs when soil is clean-cultivated presents a widespread problem in conserving the productivity of agricultural land. The problem becomes acute primarily in loam and clay soils (*9*),[2] but it occurs to a noticeable extent in some of the lighter soils as well (*3*, *8*). Deteriorated structure is found in, but not confined to eroded soil. It is an inevitable accompaniment of clean cultivation. Numerous studies (*1*) have demonstrated that when soil is subjected to clean cultivation, it tends to become more compact. The volume of large pores decreases, thereby adversely affecting the intake of water and the normal growth of roots. Where such conditions obtain, drought and nutrient deficiencies become evident despite adequate natural precipitation and the liberal use of fertilizer (*4*).

The loss of structure that accompanies clean cultivation is usually associated with various other changes in the soil. The most important of these are a decrease in organic-matter content, aggregation, nitrogen, and exchangeable bases (*1*). However, the extent to which these changes are actually responsible for poor structure or are only parallel accompaniments has been difficult to ascertain.

With the lack of critical information on the direct causes of the loss in structure, progress in preventing this deterioration has been slow. Manure and lime have been demonstrated to have beneficial effects, but in general there is no practical method for preventing loss in structure when land is clean-cultivated. Therefore farmers located on soils susceptible to such loss have to rely principally on sod crops to restore structure after each period of clean cultivation. A result of such rotating is an increase in the total acreage required for the production of clean-cultivated crops and an increase in the economic pressure on land of lower capability and greater erodibility.

In examining clean-cultivated land for clues as to the direct cause of the loss of structure, it was observed that much of the damage appeared to occur during the winter season. A laboratory study was conducted on three soils from widely separated localities to determine

[1] Received for publication September 23, 1948. Cooperative investigation of Soil Conservation Service Research and the Maryland Agricultural Experiment Station. Published as Scientific Article No. A–213, Contribution No. 2133, of the Maryland Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 352.

the effects of freezing and thawing on structure (*10*). It was found that this climatic process destroyed much of the aggregate structure when the soil was at a high moisture content. However, the damaging effect was much less at lower moisture contents. At the moisture equivalent (18 to 30 percent of moisture, depending upon the soil), freezing and thawing damaged the aggregate structure only slightly.

Solution of the problem, then, appeared to depend on the removal of excess water during the winter from the surface soil as deep as the frost line. It appeared that this might be accomplished by stimulating a natural development of drainage. The writers had found in other studies (*7*) that earthworm holes serve as drainage channels, and that the activity of earthworms during the winter improves the infiltration capacity of soil. However, on clean-cultivated land, they are usually killed during the winter by the lack of sufficient surface protection (*5*, *6*).

The present study was undertaken to determine whether the structure of clean-cultivated soil would be maintained by protecting the ground over winter, thereby decreasing depth and frequency of freezing, and stimulating the activity of the earthworms in making holes to remove excess water during the critical freezing periods.

PROCEDURE

The study was conducted on field plots located at the Maryland Agricultural Experiment Station near College Park, Md., the Ohio Agricultural Experiment Station, Wooster, Ohio, and the Northwest Experimental Farm, Holgate, Ohio. The plots had been in operation by the respective cooperating agencies for a minimum of 7 years. At each location, plots in the following crops were selected:

1. Continuous corn, stover removed;
2. Continuous corn, organic matter returned as manure or stover;
3. Corn in rotation, stover removed;
4. Wheat, followed by clover or alfalfa in rotation;
5. Continuous sod hay.

There were some differences among the locations in details of the cropping and fertilizer methods. These differences may have influenced the result somewhat, but since the purpose of the study was to determine effects of general applicability, the differences in methods were of no particular concern. The soils at Wooster and Holgate were rather uniform; at the former a glaciated, noncalcareous silt loam, and at the latter, a lacustrine clay loam. At College Park, the soil was highly variable but, fortunately, duplicate plots were available there. The soil was silt loam derived from coastal-plain deposits.

The fall-protection treatments were applied to the plots between October 15 and 23, 1947. Three adjoining areas, each approximately 6 feet long and 4 feet wide, were selected in each plot. The center one of the three areas was left uncovered, while the outer ones were covered. One was covered with hay mulch about 1-inch thick. The other was covered with strips of black asphalt roofing paper 6 inches wide with 2 inches between strips. The latter material was used in order to compare a winter covering that was relatively undecomposable with the hay mulch. However, the paper covering turned out to be

very much less protective than the mulch. In addition, the paper was blown off the plots at Holgate by a cyclone that struck during the winter. Hence no data are shown in the results for the paper covering at Holgate. The cyclone also disturbed the mulch treatment on the continuous corn plot that received organic matter. Hence, data are not given for this plot either.

The following spring, between April 5 and 29, 1948, the plots were revisited. The earthworms were counted to a depth of 8 inches on squares measuring 7 by 7 inches. Samplings were in triplicate at College Park and Wooster, and in duplicate at Holgate. Infiltration was determined in triplicate by a previously described method (*7*). Soil samples were taken for duplicate water-stability tests. This procedure likewise has been described elsewhere (*2*).

The effects of the treatments on soil porosity were also determined. Soil cores were taken with a sampler similar to that described by Baver (*1*), but of improved design to minimize structural disturbances in the soil. The cores were placed in water overnight and then clamped on a tension plate. Water was introduced from the bottom until free water appeared at the soil surface. A 25-cm. tension was then drawn for 10 minutes. The amount of water that drained was considered a measure of the pore space through which free water drained readily. The tension of 25 cm. was imposed by the design of the equipment.

RESULTS

All the data for each location are given in table 1. Since the purpose of the study was to determine effects that are broadly applicable, the results will not be discussed separately for each location. Instead, they are summarized for all locations in table 2.

Owing to the lack of some of the Holgate values, the actual averages might have been adjusted in order to make them exactly comparable. However, such adjustments would necessarily have to be somewhat theoretical and since they were unnecessary to show the main effects, they were avoided.

The various soil-structural factors (infiltration, large pores, and water-stability) and the earthworm populations showed similar trends in the response to the covering treatments. Both the paper and mulch covers affected the observed factors in the same direction, but the effect was usually greater with the mulch than with the paper.

The plots in continuous corn showed a large increase in all factors as a result of the winter covering. Where the soil was not covered over winter, the plots from which stover had been removed gave slightly lower values than the plots that had received stover or manure.

The plots that had been in rotation corn during 1947 and had young wheat on them over winter likewise showed large increases in all factors as a result of the winter covering. Where the soil was uncovered over winter, the values were not quite as low in these plots as in the continuous corn plots.

The plots that had been in rotation wheat during 1947 and had a sod cover over winter showed some increase in infiltration, large pores, and earthworms, but not in water stability as a result of the winter covering. In plots uncovered over winter, all values were larger than in those that had been in corn in 1947.

836173—49——4

TABLE 1.—*Infiltration rate, large pores, and water-stability of the soil and the earthworm population in April 1948, on agronomic plots given various overwinter protection treatments*

Cropping practice 1947	Location [1]	Overwinter protection		
		None	Paper	Mulch
Infiltration rate (in./min.): [2]				
Continuous corn	College Park	0.02	0.66	0.70
	Wooster	.00	.02	.11
	Holgate	.04		.90
Continuous corn Organic matter.[3]	College Park	.06	.31	.31
	Wooster	.01	.00	.43
Corn in rotation	College Park	.07	.49	.30
	Wooster	.02	.10	.17
	Holgate	.22		.46
Wheat in rotation	College Park	.08	.14	.04
	Wooster	.11	.23	.08
	Holgate	.15		.50
Continuous hay	College Park	.38	.52	.94
	Wooster	.58	.10	.35
	Holgate	.13		.17
Large Pores (percent): [4]				
Continuous corn	College Park	4.0	8.1	7.9
	Wooster	1.0	2.2	3.2
	Holgate	3.3		8.9
Continuous corn, organic matter.[6]	College Park	4.2	9.7	12.5
	Wooster	2.2	3.2	5.8
Corn in rotation	College Park	4.2	9.4	8.0
	Wooster	2.8	7.6	7.6
	Holgate	4.8		5.4
Wheat in rotation	College Park	7.4	8.4	7.8
	Wooster	4.4	8.8	8.0
	Holgate	6.9		9.3
Continuous hay	College Park	12.6	6.6	9.0
	Wooster	8.2	9.0	9.6
	Holgate	10.7		5.9
Water-stability (percent): [5]				
Continuous corn	College Park	35.2	33.6	41.6
	Wooster	12.6	14.3	14.0
	Holgate	41.0		48.5
Continuous corn, organic matter.[6]	College Park	42.3	29.3	35.5
	Wooster	12.4	16.4	15.8
Corn in rotation	College Park	35.5	33.7	54.7
	Wooster	32.8	42.6	55.4
	Holgate	63.2		67.0
Wheat in rotation	College Park	68.4	57.5	59.6
	Wooster	58.8	51.6	57.6
	Holgate	78.0		80.9
Continuous hay	College Park	80.4	83.6	89.1
	Wooster	70.3	83.9	72.2
	Holgate	85.8		84.7
Earthworm population (No./sq. ft.): [6]				
Continuous corn	College Park	1.5	2.9	43.6
	Wooster	2.0	6.9	19.6
	Holgate	4.9		63.7
Continuous corn, organic matter: [6]	College Park	2.9	5.9	71.0
	Wooster	7.8	11.8	13.7
Corn in rotation	College Park	6.4	6.9	70.1
	Wooster	7.8	16.7	52.9
	Holgate	14.7		32.3
Wheat in rotation	College Park	22.5	38.7	86.7
	Wooster	13.7	40.2	42.1
	Holgate	31.4		45.1
Continuous hay	College Park	25.5	30.4	45.6
	Wooster	8.8	9.8	4.9
	Holgate	38.2		100.0

[1] All plots duplicated at College Park; single at Wooster and Holgate.
[2] Each value is the average of 3 samples per plot.
[3] Data for this cropping practice available at College Park and Wooster only.
[4] Each value is the average of 2 samples per plot.
[5] Each value is the average of 2 samples per plot.
[6] Each value is the average of 3 samples per plot at College Park and Wooster; 2 at Holgate.

TABLE 2.—*Average effects of overwinter protection on soil physical factors and earthworms in April 1948*

Observation	1947 Crop	Natural winter cover	Overwinter protection		
			None	Paper	Mulch
Infiltration (inches per minute).	Continuous corn	Corn stubble	0.02	0.34	0.57
	Continuous corn, organic matter.	Corn stubble	.04	.16	.37
	Rotation corn	Young wheat	.10	.30	.31
	Rotation wheat	Clover	.11	.18	.31
	Hay	Sod	.36	.31	.49
Large pores, drained at 25 cm. tension (percent).	Continuous corn	Corn stubble	2.8	5.2	6.7
	Continuous corn, organic matter.	Corn stubble	3.2	6.4	9.2
	Rotation corn	Young wheat	3.9	8.5	7.0
	Rotation wheat	Clover	6.2	8.6	8.4
	Hay	Sod	10.5	7.8	8.2
Water stability (percent).	Continuous corn	Corn stubble	29.6	29.6	34.7
	Continuous corn, organic matter.	Corn stubble	27.4	22.8	25.6
	Rotation corn	Young wheat	43.8	49.0	59.0
	Rotation wheat	Clover	68.4	58.7	66.0
	Hay	Sod	78.9	87.6	82.0
Earthworms (number per square foot).	Continuous corn	Corn stubble	2.8	4.9	42.3
	Continuous corn, organic matter.	Corn stubble	5.4	8.8	42.4
	Rotation corn	Young wheat	9.6	11.8	51.8
	Rotation wheat	Clover	22.5	39.4	58.0
	Hay	Sod	24.2	20.1	50.2

The hay plots were least affected by the winter covering. Where the soil was uncovered over winter, the values were relatively high in these plots as compared with those for plots that had been in corn in 1947.

DISCUSSION

Values obtained from the continuous hay land that had not been covered over winter may be taken as the standard for judging good soil conditions. Where left uncovered over winter, the land that had been clean-cultivated during the summer of 1947 was in very much poorer condition than the sod. Mulching the clean-cultivated land overcame much of the difference. Its infiltration and earthworm counts were as favorable as in the sod, and the large pore space was almost as good. The water-stability of the soil was still much less than that of the sod land, but there was a definite improvement due to mulching.

The roofing-paper cover also improved the soil structural conditions on the cultivated plots but not so much as the hay mulch. This was due to the poor insulative quality of the paper as compared with the mulch; also in part, perhaps, to the organic matter that became available as food for soil organisms in the case of the mulch and not of the paper.

Mulching over winter would be practical only in gardens and on a few types of intensively operated farms. For most soils where deterioration of soil structure over winter is an important problem, more practical methods of obtaining adequate protection will have to be devised. As shown in this study, the insulative protection of winter grain is insufficient to maintain soil structure and earthworms over winter.

SUMMARY

The effect of winter protection on the structure and earthworm population of soils was determined in plots at College Park, Md.; Wooster, Ohio; and Holgate, Ohio. The soil-structure measurements used were infiltration rate, water-stability, and volume of large pores.

Clean-cultivated land left bare over winter had poorer soil structure and a lower earthworm population than land protected by sod (continuous or after wheat in rotation).

The soil structure and earthworm population were very much improved in clean-cultivated land by protecting the ground surface over winter with an insulative cover.

Winter covering had only slight effect on the structure of soil in sod.

Hay mulch was more effective than asphalt roofing paper in maintaining the structure of clean-cultivated soil over winter. Where mulching is impractical, other methods of applying the principle of winter protection to clean-cultivated land will have to be devised.

LITERATURE CITED

(1) Baver, L. D.
1948. soil physics. Ed. 2, 398 pp., illus. New York.

(2) Bryant, J. C., Bendixen, T. W., and Slater, C. S.
1948. measurement of the water-stability of soils. Soil Sci. 65: 341–345.

(3) Hester, J. B., and Shelton, F. A.
1937. the results of long-time fertilization and cropping practices upon the chemical and physical composition of coastal plain soils. Soil Sci. Soc. Amer. Proc. (1936) 2: 201–205.

(4) Hoffer, G. N.
1945. fertilized corn plants require well-ventilated soils. Better Crops With Plant Food. 29: 6.

(5) Hopp, H., and Linder, P. J.
1947. a principle for maintaining earthworms in farm soils. Science 105: 663.

(6) ———
1948. the ecology of earthworms in farm soils. Soil Sci. Soc. Amer. Proc. (1947) 12: 503–507.

(7) ——— and Slater, C. S.
1948. influence of earthworms on soil productivity. Soil Sci. 66: 421–428.

(8) Kolodny, L., and Neal, O. R.
1941. the use of micro-aggregation or dispersion measurements for following changes in soil structure. Soil Sci. Soc. Amer. Proc. (1940) 6: 91–95.

(9) Page, J. B., and Willard, C. J.
1947. cropping systems and soil properties. Soil Sci. Soc. Amer. Proc. (1946) 11: 81–88.

(10) Slater, C. S., and Hopp, H.
1949. the action of frost on the water-stability of soils. J. Agr. Res. 78.

VARIABILITY AND CORRELATION IN A COTTON BREEDING PROGRAM[1]

By G. N. STROMAN

Agronomist, New Mexico Agricultural Experiment Station

INTRODUCTION

The cotton breeder is always concerned with two factors: The variability of the many characters with which he is working and the correlation between these characters. His breeding material must vary enough to include at least the potentialities for all the desired characters as well as for all other favorable characters of yield and quality. As his work progresses, he narrows the variability of the essential characters of quality, such as fiber strength, length, uniformity, fineness, and maturity, and of certain characters of yield, such as lint percentage, lint index, and boll weight. Frequently, characters seem to be correlated. This may present a problem to the breeder; because if a desirable character is associated with an undesirable one, selection for one without the other is difficult.

This article reports the biometrical relationships of certain characters used in the cotton breeding program at the New Mexico Agricultural Experiment Station. The purpose in determining these relationships was to discover how the characters in the breeding material are related, and to measure, from year to year, any changes in correlation.

PREVIOUS WORK ON CORRELATION OF COTTON CHARACTERS

The relationships between characters in upland cotton have been studied by many workers. Those reported vary greatly according to the material used and the place where the cotton was grown.

The yield of lint cotton from each plant was found by Stroman (*23*)[2] to depend on weight of bolls, number of five-lock bolls, number of four-lock bolls, number of vegetative branches, number of fruiting branches, height of plant, and lint percentage. The multiple correlation between yield of lint per plant and the other characters varied, in the several lines studied, from 0.96 to 0.99. The partial correlation coefficients between the characters indicated that number of bolls, weight of bolls, and height of plant were more important to yield than were the other characters. The height-yield relationship was negative. The weight of boll was negatively related to

[1] Received for publication July 9, 1948.

[2] Italic numbers in parentheses refer to Literature Cited, page 362.

number of bolls. Hancock (*8*) reported significant negative correlations between height of plant and both number of fruiting branches and number of bolls. Buie (*3*) reported a multiple correlation of 0.86 between first-picking yield and date of first flower, flowering period, boll period, and first open boll. Harrison (*9*) reported, in Durango cotton, coefficients involving boll period, lint length, lint index, number of seed per boll, and weight of seed. Most of his correlations were significant.

Kearney (*13*, *14*), Humphrey (*11*), Hodson (*10*), Dunlavy (*5*), Martin and Mason (*17*), Stroman (*22*), and Patel (*20*), have reported relationships involving yield characters and length of lint. Most of these relationships were negative and significant. Stroman (*24*) reported relationships in the F_3 generation of *G. hirsutum*×*G. barbadense* cross. The correlations, some of which were significant, varied greatly in the different lines. The characters used were lint percentage, lint index, boll weight, and length of lint. Some relationships involving fiber length and color in Indian cotton have been reported by Kearney (*14*) and Kottur (*16*). Nanjundayya and Ahmad (*19*) reported a relationship between mean strength and weight per unit length which varied from 0.735 to 0.835, and one between length and strength of fiber from −0.15 to −0.30 (n=45).

Gulati and Ahmad (*7*) studying maturity of cotton fibers in relation to other characters, found the following significant correlation coefficients:

Characters:	r
Mean fiber strength and percent mature hairs	0.72
Mean fiber strength and percent half mature hairs	−.46
Mean fiber strength and percent immature hairs	−.71
Fiber weight per inch and percent mature hairs	.59
Fiber weight per inch and percent immature hairs	−.60
Neps in yarn and percent immaturity of fibers	.81
Fiber maturity and spinning performance (long cotton)	.56

Koshal and Ahmad (15), studying swollen fiber diameter, obtained the following coefficients:

Characters:	r
Swollen fiber diameter and fiber weight per inch	0.746
Swollen fiber diameter and standard hair weight	.839
Swollen fiber diameter and spinning value	−.800

Conrad and Berkley (*4*) found a correlation of 0.754 between X-ray arc length and number of 60.0 skein strength; they also found a multiple correlation coefficient of 0.91 between X-ray arc length and characters of fiber length, fineness, and maturity.

Turner and Venkataraman (*29*) investigated the relationship of the raw fiber characters and the spinning value of two series of Indian cottons. The partial correlations they found indicated that three raw fiber characters—fiber length, fiber weight per inch, and fiber width—were associated with spinning value. One of the coefficients they reported between fiber length and yarn strength was 0.87. Underwood (*30*), working with Egyptian cotton, reported a correlation coefficient of 0.77 between these two characters. Ahmad and Navkal (*1*), working with Indian cotton, reported significant correlation coefficients between all of the following characters: Spinning

value, mean fiber length, fiber weight per inch, and fiber length irregularity.

Turner (*28*) has reported a coefficient of −0.305 between count strength product and mean fiber strength. Barrett (*2*) reported correlation coefficients of −0.41 and −0.61 between yarn strength and fiber strength. Hutchinson and Govande (*12*) found a significant coefficient of −0.58 between the highest standard count and swollen fiber diameter, and one of −0.597 between the highest standard count and hair weight.

Webb and his associates (*32, 33, 34, 35*) have, in the last few years, completed preliminary reports on the biometrical relationships of the raw fiber characters and spinning performance of American cotton. The raw fiber characters are: Strength of fiber, maturity of fiber, length variability, fineness, upper half mean or three-fourths point, and grade.

MATERIALS AND METHODS

The individuals composing the populations were a mixture of related families in the regular breeding program at the New Mexico Agricultural Experiment Station. The families originated from early Acala selections, from general fields, and from crosses between certain related strains. Some of the families may have been derived from natural hybrids of more or less closely related strains, but no wide-cross progenies were included. Each year, the population was limited to those individuals given complete laboratory tests.

The characters used to indicate lint yield were: Lint percentage, which is obtained by dividing the weight of the lint in a sample by the weight of the lint and the seed; lint index, which is the percentage of lint multiplied by the weight of 100 seeds and then divided by the percentage of seed; and boll weight, which is the average weight of lint in a boll, in grams, from a 15-boll sample. In 1937, two other characters were also used—total bolls per plant and total fibers, or the total weight of fibers in a sample as measured by the Pressley Fiber Sorter.

Characters indicating fiber length were: Classer's length, as determined by a licensed government classer; the percentage of fibers from a 10-seed sample which measures 1⅛ inches or more in length, and which will be referred to in this paper as percentage 1⅛-inch (plus) fibers; and the three-fourths point, determined by use of a Pressley Automatic Fiber Sorter, which also was used to determine the percentage of 1⅛-inch (plus) fibers and the mean length of lint.

Uniformity of lint length was determined by the standard deviation of all the fibers in a sample. The percentage of 1⅛-inch (plus) fibers is also a measure of uniformity.

Strength of fiber is measured on a Pressley Strength Tester. It is reported as an index number, derived by dividing the pounds required for breakage by the weight of the broken fibers, which are of a standard length. In 1944, the strength index-percentage of standard sample was used. It is obtained by breaking a standard sample in every fifth sample.

Fiber diameter is obtained by measuring from 50 to 100 fibers, which have been swollen in an 18-percent sodium hydroxide solution,

with a micrometer eyepiece in a microscope. The eyepiece and objectives used give readings which are two-thirds of the swollen fiber size, or approximately the natural diameter of the fiber.

The variability in fiber diameter is expressed by the standard deviation of 50 to 100 fibers measured for fiber diameter. This character was used only in the calculation of simple correlation coefficients in 1944.

Fiber maturity, which was also used only in 1944, was measured by swelling fibers in an 18-percent sodium hydroxide solution and counting the mature fibers in a 100-fiber sample.

The tables indicate which characters were used each year. The number of individuals composing the populations were as follows: 1937, 162; 1940, 151; 1944, 441; 1945, 143; and 1946, 313.

Simple correlation coefficients were calculated each year between the characters. From these coefficients, multiple correlation coefficients were calculated (*28*). The partial correlation coefficients between all characters were calculated by multiple regression coefficients (*34*, *23*, *6*).

THE DATA

VARIABILITY

The mean, standard deviation, and the coefficient of variability of all the characters used in this study are found in table 1. The variability data are included mainly to show levels of the characters used in calculating the correlation coefficients. In passing, however, it is well to note a few points that may be interesting from the standpoint of practical cotton breeding. It is shown that, in 1937, the mean lint percentage of the progenies was 39.99 percent; the lint index was 9.08; the classer's length of lint was 36.51 thirty-seconds inch; the boll weight was 3.27 grams of lint. These had been built up over a long period. During this time, the percentage of 1⅛-inch (plus) fibers had been built up by the breeding program to 37.07 percent. The first lines tested for this character ranged between about 5 percent to practically 30 percent. As the work progressed and the testing for percentage 1⅛-inch (plus) fibers became more extensive, progenies with higher and higher percentages were developed. The lint percentage, lint index, classer's length, and boll weight decreased somewhat through the period shown, but while this occurred, the percentage of 1⅛-inch (plus) fibers increased to 52.01 percent in 1946. The fiber diameter of 13.93 microns in 1940 was a desirable diameter, but in 1944 it was coarser, 15.42 microns, and in 1946 it had decreased nearly 1 micron.

From 1937 to 1946 the variability, as measured by the standard deviation and coefficient of variability, was recorded for most characters. However, the range of most characters still gave a rather wide amount of variation. In cotton breeding, the variability must be wide enough to indicate the expression of as many genes as possible and it can be narrowed only through the testing, hybridization, and selection of the desirable progenies and individuals. Because certain characters are influenced by seasonal conditions, the degree of improvement can be measured only over long periods.

TABLE 1.—*Mean, Standard Deviation, and Coefficient of Variation of Each Character Used Each Year in Calculation of Correlation Coefficients*

	1937	1940	1944	1945	1946
Means:					
Lint percentage	39.99	36.10	37.37	34.90	37.15
Lint index	9.08	8.25	8.28	7.81	8.26
Boll weight (grams)	3.27	3.14	----	2.94	2.93
Total fibers (milligrams)	353.70	----	----	----	----
Total bolls per plant (number)	47.20	----	----	----	----
Classer's length (1/32 inch)	36.51	----	35.63	36.17	35.95
Percentage 1⅛-inch (plus) fibers	37.07	43.44	50.68	46.38	52.01
Mean length (inches)	----	----	----	.99	1.02
Three-fourths point (inches)	----	----	----	----	1.13
Length variability	----	----	----	25.32	23.88
Strength index	----	----	7.12	6.61	6.80
Strength index percent standard	----	----	102.08	----	----
Fiber diameter (microns)	----	13.93	15.42	14.82	14.46
Standard deviation of fiber diameter	----	----	2.76	----	----
Fiber maturity (percentage)	----	----	91.24	----	----
Standard deviations:					
Lint percentage	2.22	1.78	1.93	1.82	1.59
Lint index	.91	.69	.64	.69	.70
Boll weight	.38	.34	----	.51	.29
Total fibers	49.06	----	----	----	----
Total bolls per plant	13.79	----	----	----	----
Classer's length	.80	----	1.25	.68	.78
Percentage 1⅛-inch (plus) fibers	9.46	12.00	13.71	10.92	11.39
Mean length	----	----	----	.04	.05
Three-fourths point	----	----	----	----	.05
Length variability	----	----	----	2.19	2.39
Strength index	----	----	.99	.51	.56
Strength index percentage standard	----	----	7.59	----	----
Fiber diameter	----	.61	.92	.55	.54
Standard deviation of fiber diameter	----	----	.54	----	----
Fiber maturity	----	----	5.19	----	----
Coefficients of variability (percent):					
Lint percentage	5.5	4.93	5.18	5.22	4.29
Lint index	10.1	8.36	7.79	8.79	8.53
Boll weight	11.6	10.83	----	17.29	9.86
Total fibers	13.9	----	----	----	----
Total bolls per plant	29.2	----	----	----	----
Classer's length	2.2	----	3.51	1.89	2.16
Percentage 1⅛-inch (plus) fibers	25.5	27.62	27.05	23.54	21.90
Mean length	----	----	----	4.08	4.47
Three-fourths point	----	----	----	----	4.43
Length variability	----	----	----	8.64	9.99
Strength index	----	----	13.84	7.78	8.19
Strength index percentage standard	----	----	7.43	----	----
Fiber diameter	----	4.31	5.96	3.73	3.71
Standard deviation of fiber diameter	----	----	19.46	----	----
Fiber maturity	----	----	5.68	----	----
Populations	162	151	441	143	313

RELATIONSHIPS OF THE CHARACTERS

Tables 2 to 7, inclusive, show the simple and partial correlations between all possible combinations of characters. The discussion is limited to the partial correlations, because these show the relationship between two characters and eliminate the influence of the fluctuations of the other characters.

The data show that the relationship of certain characters may not be the same each year. Different climatic and soil conditions of the different years certainly influence the expression of certain genes. Selection also contributes some influence on the relationships. However, certain correlations remain about the same from year to year.

Characters of Yield

Lint percentage and lint index are strongly correlated each year.

The classic example of correlation in cotton characters, the negative correlation between lint percentage and length of lint, occurred

TABLE 2.—*Simple (S) and Partial (P) Correlation Coefficients of Lint Percentage With Other Characters*

Characters	1937		1940		1944		1945		1946	
	S	P	S	P	S	P	S	P	S	P
Lint index	0.76	0.61	0.61	0.49	0.38	0.46	0.69	0.64	0.59	0.56
Percentage of 1⅛-inch (plus) fibers	−.36	−.32	−.44	−.35	−.57	−.44	−.02	−.02	−.21	−.05
Classer's length lint	.16	−.02	.....	.....	−.34	−.14	−.02	−.14	−.22	−.14
Strength index	.....	.....	.....	.....	−.37	−.25	−.21	−.09	−.24	−.18
Fiber diameter	.....	.....	.10	−.12	−.03	−.06	.22	.06	.14	−.004
Mean length lint	.....	.....	.....	.....	.....	.....	−.10	.01	−.18	−.08
Lint length variability	.....	.....	.....	.....	.....	.....	.19	.12	.02	−.04
Boll weight	.60	.13	.42	.09	.....	.....	.43	.34	.26	.001
¾ point	.....	.....	.....	.....	.....	.....	.....	.....	−.17	.06
Total fibers	.19	−.25	.....	.....	.....	.....	.....	.....	.....	.....
Least significant *r*:										
5-percent level	.15	.16	.16	.16	.09	.09	.16	.17	.11	.11
1-percent level	.20	.20	.21	.21	.12	.12	.21	.22	.14	.15

TABLE 3.—*Simple (S) and Partial (P) Correlation Coefficients of Lint Index With Other Characters*

Characters	1937		1940		1944		1945		1946	
	S	P	S	P	S	P	S	P	S	P
Lint percentage	0.76	0.61	0.61	0.49	0.38	0.46	0.69	0.64	0.59	0.56
Percentage of 1⅛-inch (plus) fibers	−.19	.10	−.30	.05	−.04	.11	.16	.13	.04	−.02
Classer's length unit	.19	.10	.....	.....	.05	.04	.14	.17	−.04	.02
Strength index	.....	.....	.....	.....	.17	.19	−.14	−.05	−.11	.04
Fiber diameter	.....	.....	.19	.11	.32	.20	.17	.06	.20	.11
Mean length lint	.....	.....	.....	.....	.....	.....	.11	−.09	.09	.15
Lint length variability	.....	.....	.....	.....	.....	.....	.03	−.13	−.11	.05
Boll weight	.73	.43	.53	.37	.....	.....	.34	.01	.45	.35
¾ point	.....	.....	.....	.....	.....	.....	.....	.....	.04	−.09
Total fibers	.45	.37	.....	.....	.....	.....	.....	.....	.....	.....
Least significant *r*:										
5-percent level	.15	.16	.16	.16	.09	.09	.16	.17	.11	.11
1-percent level	.20	.20	.21	.21	.12	.12	.21	.22	.14	.15

TABLE 4.—*Simple (S) and Partial (P) Correlation Coefficients of Percentage of 1⅛ + Fibers With Other Characters*

Characters	1937		1940		1944		1945		1946	
	S	P	S	P	S	P	S	P	S	P
Lint percentage	−0.36	−0.32	−0.44	−0.35	−0.57	−0.44	−0.02	−.02	−0.21	0.05
Lint index	−.19	.10	.....	.....	−.04	.11	.16	.13	.04	−.02
Classer's length lint	−.05	−.01	.....	.....	.46	.27	.31	−.60	.39	.04
Strength index	.....	.....	.....	.....	.43	.20	.26	.04	.17	.08
Fiber diameter	.....	.....	−.27	−.24	.18	−.05	−.13	.10	−.02	.04
Mean length lint	.....	.....	.....	.....	.....	.....	.90	.96	.91	.57
Lint length variability	.....	.....	.....	.....	.....	.....	−.20	.81	−.26	.17
Boll weight	−.17	−.002	−.30	−.11	.....	.....	.01	.04	.07	.02
¾ point	.....	.....	.....	.....	.....	.....	.....	.....	.87	.26
Total fibers	−.01	.01	.....	.....	.....	.....	.....	.....	.....	.....
Least significant *r*:										
5-percent level	.15	.16	.16	.16	.09	.09	.16	.17	.11	.11
1-percent level	.20	.20	.21	.21	.12	.12	.21	.22	.14	.15

TABLE 5.—*Simple (S) and Partial (P) Correlation Coefficients of Classer's Length of Lint With Other Characters*

Characters	1937		1944		1945		1946	
	S	P	S	P	S	P	S	P
Lint percentage	0.16	−0.02	−0.34	−0.14	−0.02	−0.14	−0.22	−0.14
Lint index	.19	.10	.05	.04	.14	.17	−.04	.02
Percentage of 1⅛-inch (plus) fibers	−.05	−.01	.46	.27	.31	−.60	.39	.04
Strength index			.34	.06	.13	.002	.05	−.05
Fiber diameter			.34	.20	−.15	−.15	−.03	−.01
Mean length lint					.45	.68	.39	.06
Lint length variability					−.07	.61	−.12	.001
Boll weight	.17	.05			.21	.22	−.004	−.004
¾ point							.37	.04
Total fibers	.03	−.07						
Least significant *r*:								
5-percent level	.15	.16	.09	.09	.16	.17	.11	.11
1-percent level	.20	.20	.12	.12	.21	.22	.14	.15

TABLE 6.—*Simple (S) and Partial (P) Correlation Coefficients of Strength Index With Other Characters*

Characters	1944		1945		1946	
	S	P	S	P	S	P
Lint percentage	−0.37	−0.25	−0.21	−0.09	−0.24	−0.18
Lint index	.17	.19	−.14	−.05	−.11	.04
Percentage of 1⅛-inch (plus) fibers	.43	.20	.26	.04	.17	.08
Classer's length lint	.34	.06	.13	.002	.05	−.05
Fiber diameter	.45	.41	−.31	−.24	−.14	−.12
Mean length lint			.27	.02	.15	−.04
Lint length variability			−.13	.01	−.09	−.06
Boll weight			−.03	.05	−.07	−.02
¾ point					.13	.01
Least significant *r*:						
5-percent level	.09	.09	.16	.17	.11	.11
1-percent level	.12	.12	.21	.22	.14	.15

TABLE 7.—*Simple and Partial Correlation Coefficients of Fiber Diameter With Other Characters*

Characters	1940		1944		1945		1946	
	S	P	S	P	S	P	S	P
Lint percentage	0.10	−0.12	−0.03	0.06	0.22	0.06	0.14	−0.004
Lint index	.19	.11	.32	.20	.17	.06	.20	.11
Percentage of 1⅛-inch (plus) fibers	−.27	−.24	.18	−.05	−.13	.10	−.02	.04
Classer's length lint			.34	.20	−.15	−.15	−.03	−.01
Strength index			.45	.41	−.31	−.24	−.14	−.12
Mean length lint					−.18	.10	−.03	−.11
Lint length variability					.16	.13	−.01	−.08
Boll weight	.21	.10			.04	−.001	.18	.09
¾ point							.0003	.08
Least significant *r*:								
5-percent level	.16	.16	.09	.09	.16	.17	.11	.11
1-percent level	.21	.21	.12	.12	.21	.22	.14	.15

only twice when length of lint was measured by classer's length, mean length, or three-fourths point. The partial correlation coefficient between lint percentage and classer's length was significant in 1944 and 1946. When length was measured by percentage of 1⅛-inch (plus) fibers, which is more a measure of fiber length uniformity than of length, the partial negative correlation between fiber length and percentage was significant in 1937, 1940, and 1944, but not in 1945 and 1946.

This may indicate that, as the individuals with a high percentage of lint and a high percentage of 1⅛-inch (plus) fibers were selected along with the other desirable characters, the correlation has been broken, and that the genes of both lint percentage and percentage of 1⅛-inch (plus) fibers in the breeding material in 1945 and 1946 were independent.

In 3 of the 4 years when boll weight was tested, the positive partial correlation coefficients between it and lint index were significant. Outside of that and two other correlations, boll weight is independent of the other characters, according to the data. In one population, it was correlated with classer's length; in another, with lint percentage.

Characters of Lint Length

The mean length of lint and lint length variability were used only in 1945 and 1946. They were correlated with each other and with the percentage of 1⅛-inch (plus) fibers.

Percentage of 1⅛-inch (plus) fibers was also correlated, in 1946, with the three-fourths point. In 1944, this character was positively correlated with the classer's length, but in 1945, the two were negatively correlated. The 1945 relationship is not easily explained, because the percentage of 1⅛-inch (plus) fibers should be positively correlated with classer's length. Classer's length is influenced by fiber strength as well as length, and in 1945, the classer's length may have been based on a finding that the longer fibers were weaker than the shorter fibers. But if this were the case it was not confirmed by a correlation between percentage of 1⅛-inch (plus) fibers and strength index.

In 1946, the classer's length was not associated significantly with any of the other characters, as shown by the partial correlation coefficients in table 5. The same table shows that, in 1945, the character was negatively correlated with percentage of 1⅛-inch (plus) fibers, and positively with mean length, length variability, and boll weight. In 1944, it was associated with percentage of 1⅛-inch (plus) fibers and with fiber diameter.

Strength Index

Table 6 shows that the strength index was not strongly correlated with any of the characters used. Significant, though small, negative correlations were found between this character and lint percentage in 1944 and 1946. It was positively correlated with fiber diameter in 1944 and negatively in 1945.

Fiber Diameter

There were no significant partial correlation coefficients between fiber diameter and the other characters in 1946, as shown in table 7. In 1945, fiber diameter and strength index were negatively correlated,

and in 1944, they were positively correlated. The character was negatively correlated with percentage of 1⅛-inch (plus) fibers in 1940, but not in other years. In 1944, it was positively correlated with lint index and classer's length.

MULTIPLE CORRELATION COEFFICIENTS

Table 8 shows the multiple correlation for all the characters used each year. None of the coefficients are very large. This is an encouraging sign for the breeder, because strong associations, where some are positive and some are negative, are obstacles in the breeding program.

DISCUSSION

The data show that correlations between the characters studied in the cotton breeding material were not always consistent from one year to another. If a generalization may be drawn from this study, it is that no correlations are so strong as to preclude unaccounted-for variation. By utilizing this variation, the cotton breeder should be able to combine all the desirable characters found in the material at hand.

TABLE 8.—*The multiple correlation coefficients (R) and coefficient of determination (R^2) involving various characters*

Characters	1937		1940		1944		1945		1946	
	R	R^2	R	R^2	R	R^2	R	R^2	R	R^2
Lint percentage	0.80	0.65	0.68	0.46	0.71	0.51	0.76	0.58	0.66	0.44
Lint index	.86	.74	.69	.48	.55	.31	.72	.53	.69	.48
Percentage of 1⅛-inch (plus) fibers	.38	.14	.50	.25	.67	.45	.97	.94	.93	.87
Classer's length lint	.21	.04	------	------	.55	.31	.75	.56	.43	.18
Strength index	------	------	------	------	.67	.44	.41	.17	.31	.09
Strength index percentage standard	------	------	------	------	.41	.17	------	------	------	------
Fiber diameter	------	------	.33	.11	.60	.36	.39	.16	.28	.08
Mean length lint	------	------	------	------	------	------	.98	.96	.96	.92
Lint length variability	------	------	------	------	------	------	.88	.78	.74	.55
Boll weight	.74	.54	.56	.32	------	------	.50	.25	.47	.22
¾ point	------	------	------	------	------	------	------	------	.92	.85
Total fibers	.53	.28	------	------	------	------	------	------	------	------
Total bolls per plant	.23	.05	------	------	------	------	------	------	------	------

Correlation coefficients show a relationship between two characters. Each of these characters may be the result of the expression of several genes. Significant correlations between characters may be caused by multiple factors, some of which may influence both characters in combination with other genes. Also, one or more of the genes involved in the expression of one character might be linked with one or more of the genes which cause the expression of the other character. The relationships given here represent the available material of the breeding program for each year. The genotypes involved in 1 year therefore may be different from the genotypes involved for the other years. Furthermore, genes express themselves according to their environment, which might cause relationships measured by the correlation coefficient to be different from year to year. Unpublished data at this station destroyed by fire in 1937, indicated that variety, soil, fertilizer treatment, and irrigation treatments influence the relationship of the characters of the cotton plant.

SUMMARY

The simple, partial, and multiple correlation coefficients were determined between characters in the cotton breeding material of the New Mexico Agricultural Experiment Station for 5 years. These characters were: Lint percentage, lint index, boll weight, classer's length, percentage of 1⅛-inch (plus) fibers, mean length, length variability, three fourths point, strength index, and fiber diameter. The material used was related selected progenies and these were given the complete laboratory tests.

Lint percentage and lint index were positively correlated each year. Other significant correlations varied as to degree and relationship for the different years.

The wide variability, even with the correlations, offers the possibility of combining all the desired characters of yield and quality into one strain. This necessitates the use of a larger number of individuals from selected line-bred or hybrid material.

LITERATURE CITED

1. Ahmad, N., and Navkal, Harerao.
   1933. Fibre-length irregularity in cotton. Ind. Cent. Cot. Com. Tech. Lab. Tech. Bul. Ser. B. No. 16.
2. Barrett, N. W.
   1929. The intrinsic strength of cotton. Jour. Text. Inst. 20: T71–T72.
3. Buie, T. S.
   1929. Fruiting habit of the cotton plant. S. C. Agr. Expt. Sta. Bul. 261.
4. Conrad, Carl M., and Berkley, Earl E.
   1938. Some possibilities and limitations of X-ray methods for measuring the strength of raw cotton. Textile Research 8: 341–352.
5. Dunlavy, H.
   1923. Correlation of characters in Texas cotton. Jour. Amer. Soc. Agron. 15: 444–448.
6. Ezekiel, Mordecai.
   1930. Methods of correlation analysis. John Wiley & Sons. 427 p.
7. Gulati, A. N., and Ahmad, N.
   1935. Fibre-maturity in relation to fibre and yarn characteristics of Indian cotton. Ind. Cent. Cot. Com. Tech. Lab. Tech. Bul. Ser. B. No. 20.
8. Hancock, N. I.
   1941. Relative growth rate of the main stem of the cotton plant and its relationship to yield. Jour. Amer. Soc. Agron. 33: 590–602.
9. Harrison, George J.
   1931. Metaxenia in cotton. Jour. Agr. Res. 42: 521–544.
10. Hodson, E. A.
   1920. Correlation of certain characters in cotton. Ark. Agr. Expt. Sta. Bul. 169.
11. Humphrey, L. M.
   1940. Effects of inbreeding cotton with special reference to staple length and lint percentage. Ark. Agr. Expt. Sta. Bul. 387.
12. Hutchinson, J. B., and Govande, G. K.
   1938. Cotton botany and the spinning value and hair properties of cotton lint. Ind. Jour. Agr. Sci. 8: 21–47.

13. KEARNEY, T. H.
1923. SEGREGATION AND CORRELATION OF CHARACTERS IN AN UPLAND-EGYPTIAN COTTON HYBRID. U. S. D. A. Department Bul. 1164.
14. ———
1926. CORRELATIONS OF SEED, FIBRE, AND BOLL CHARACTERS IN COTTON. Jour. Agr. Res. 33: 781–796.
15. KOSHAL, R. S., and AHMAD, N.
1939. THE DETERMINATION OF THE SWOLLEN DIAMETER OF COTTON FIBERS—EFFECT OF SIZE OF SAMPLE ON THE SIGNIFICANCE AND RELIABILITY OF THE RESULTS. Ind. Cent. Cot. Com. Tech. Lab. Tech. Bul. Ser. B. No. 27.
16. KOTTUR, G. L.
1923. STUDIES IN INHERITANCE IN COTTON. I. History of a cross between *Gossypium herbaceum and Gossypium neglectum*. Ind. Dept. Agr. Men. Bot. Ser. 12: 71–133.
17. MARTIN, R. F., and MASON, T. G.
1924. PRELIMINARY REPORT ON THE IMPROVEMENT OF AMERICAN COTTON NORTHERN NIGERIA. Nigeria Agr. Dept. Ann. Bul. 3: 41–44.
18. MCCLELLAND, C. K., and NEELY, J. WINSTON.
1931. THE ORDER RATE AND REGULARITY OF BLOOMING IN THE COTTON PLANT. Jour. Agr. Res. 42: 751–763.
19. NANJUNDAYYA, C., and AHMAD, N.
1938. STUDIES IN THE VARIATION OF STRENGTH AND WEIGHT PER INCH WITH GROUP LENGTH OF COTTON FIBERS. Ind. Cent. Cot. Com. Tech. Lab. Tech. Bul. Ser. B. No. 24.
20. PATEL, M. S.
1922. THE CONNECTION BETWEEN SEED WEIGHT AND LINT WEIGHT IN COTTON. Agr. Jour. India. 17: 204–205.
21. SNEDECOR, GEORGE W.
1946. STATISTICAL METHODS. 4th ed. The Iowa State College Press. 485 p.
22. STROMAN, G. N.
1925. BIOMETRICAL STUDIES OF LINT AND SEED CHARACTERS IN COTTON. Tex. Agr. Expt. Sta. Bul. 332.
23. ———
1930. BIOMETRICAL RELATIONSHIPS OF CERTAIN CHARACTERS IN UPLAND COTTON. Jour. Amer. Soc. Agron. 22: 327–340.
24. ———
1932. CORRELATION OF CERTAIN LINT CHARACTERS IN COTTON AND THEIR PRACTICAL APPLICATION. Jour. Agr. Res. 44: 523–527.
25. ———
1934. COTTON BREEDING INVESTIGATIONS, 1928–32. N. Mex. Agr. Expt. Sta. Bul. 217.
26. ———
1935. PROCEDURE FOR RAPID CALCULATION OF MULTIPLE CORRELATION COEFFICIENTS. Jour. Agr. Res. 50: 59–69.
27. ———
1938. IMPROVED STRAINS OF ACALA COTTON FOR NEW MEXICO. N. Mex. Agr. Expt. Sta. Bul. 256.
28. TURNER, A. J.
1928. THE FOUNDATIONS OF YARN STRENGTH AND YARN EXTENSION. Part I. The general problem; Part II. The relation of yarn strength to fibre-strength. Ind. Cent. Cot. Com. Tech. Lab. Tech. Bul. Ser. B. No. 7.
29. TURNER, A. J., and VENKATARAMAN, V.
1933. THE FOUNDATIONS OF YARN STRENGTH AND YARN EXTENSION. Part V. The prediction of the spinning value of a cotton from its fibre—properties. Ind. Cent. Cot. Com. Tech. Lab. Tech. Bul. Ser. B. No. 17.
30. UNDERWOOD, C.
1935. THE RELATIONSHIP BETWEEN SOME PROPERTIES OF COTTON HAIRS AND THE SPINNING QUALITY OF THE COTTONS. Jour. Text. Inst. 26: T309–T335.

31. WALLACE, H. A., and SNEDECOR, GEORGE W.
1931. CORRELATION AND MACHINE CALCULATION. Iowa State College Official Publication 30 (4).
32. WEBB, R. W., and RICHARDSON, H. B.
1945a. RELATIONSHIPS BETWEEN PROPERTIES OF COTTON FIBERS AND STRENGTH OF CARDED YARNS. Office Marketing Service War Food Administration. U. S. D. A. (Mimeographed.)
33. ———
1945b. RELATIONSHIPS OF COTTON FIBER PROPERTIES TO STRENGTH AND ELONGATION OF TIRE CORD. Office Marketing Service War Food Administration. U. S. D. A. (Processed)
34. ———
1946a. RELATIONSHIPS BETWEEN PROPERTIES OF COTTON FIBERS AND APPEARANCE OF CARDED YARNS. Production and Marketing Administration. U. S. D. A. (Processed)
35. ———
1946b. RELATIONSHIPS BETWEEN PROPERTIES OF COTTON FIBERS AND PERCENTAGES OF WASTES ASSOCIATED WITH THE MANUFACTURE OF CARDED YARNS. Production and Marketing Administration U. S. D. A. (Processed.)

FUSARIA ASSOCIATED WITH MIMOSA WILT, SUMAC WILT, AND PINE PITCH CANKER[1]

By WILLIAM C. SNYDER,[2] *associate professor of plant pathology, University of California*; E. RICHARD TOOLE, *associate pathologist*, and GEORGE H. HEPTING, *senior pathologist, Division of Forest Pathology,*[3] *Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture*

INTRODUCTION

In recent years several new tree diseases caused by species of *Fusarium* have been described. At times other Fusaria in addition to the species responsible for the diseases have been found closely associated with the pathogens. The Asheville, N. C., laboratory of the Division of Forest Pathology has worked with 6 of the 10 species of *Fusarium* recognized by Snyder and Hansen (*13*, *14*, *16*)[4] and with a number of formae in connection with studies on the mimosa wilt (*5*, *18*), the sumac wilt (*20*), and the pitch canker disease of pine (*8*). This paper is intended to clarify the taxonomy and nomenclature of the Fusaria encountered in studying these 3 diseases and presents information of value in making identifications and in understanding the variability encountered in natural clones[5] and laboratory mutants within a given species.

In the final assignment of names to the various species studied in this work, the system of classification and nomenclature of Snyder and Hansen (*13*, *14*, *16*) is used. In this system the species is based upon morphology and the forma in a trinomial denotes pathogenicity to a certain host or hosts. The extreme variability within some species, and even within some formae, with regard to spore size, pigmentation, conidial formation and septation, and gross cultural appearance among natural clones as well as subsequent mutants and among cultures exposed to different environmental conditions requires adoption of such a system if an endless number of species names—one applied to each variant—is to be avoided. Since this wide range of variation can be obtained among clones from single-spore cultures derived from a single pure culture of an isolate from nature, the naming of such

[1] Received for publication August 13, 1948.

[2] On temporary assignment to Division of Forest Pathology.

[3] In cooperation with Southeastern Forest Experiment Station, U. S. Forest Service, Asheville, N. C.

[4] Italic numbers in parentheses refer to Literature Cited, p. 381.

[5] The term "natural clone" denotes any form isolated directly from nature and is synonymous with Miller's "wild type" (*9*) where the latter is used in this sense. The term is used in opposition to "laboratory clone," the latter denoting mutants or growth types which develop in the course of laboratory culture practice.

variants as separate species is untenable. No spore measurements for the three formae being named are given in this paper, because their spore dimensions fall within the common ranges given by Wollenweber and Reinking (*22*) for the respective species.

IDENTIFICATION IN FUSARIUM

Species identification of an unknown *Fusarium* required that its morphologic features be determined, since these should constitute the sole basis on which the species are recognized. Since the sporulation of a *Fusarium* in nature is usually in the form of sporodochia, the attempt to determine *Fusarium* species directly from field specimens necessarily restricts a morphologic study largely to the sporodochia themselves. Often the macroconidia so obtained in nature reflect unfavorable influences of environment, substrate, other competitive organisms, and undermaturity or overmaturity. Furthermore, in nature more than one *Fusarium* species may invade a given substrate and form reproductive organs on the same tissues. Because of these facts, it is imperative that all efforts to identify *Fusarium* species be preceded by their transfer to a suitable culture medium by the single-spore method and that they be cultivated under conditions best suited to the development of the reproductive structures used in taxonomy.

PURE-CULTURE METHOD

Every culture referred to in this paper was initiated by a single spore transferred to a potato-dextrose-agar slant. Single-spore cultures were prepared either directly from spores found in nature, for example, on bark, or from conidia produced after a few days on tissue platings made from invaded plant parts. Ordinarily 10 single-spore cultures were prepared from each specimen; but, if the supply of conidia was inadequate, single-hyphal-tip cultures were prepared instead. Dilution plates of spores and of hyphal fragments were made on water agar.

During the summer and fall the cultures were laid on tables near a window, where they were exposed during their entire development to diffuse but not direct sunlight. Under these conditions most of the Fusaria included in this study sporulated normally; however, in some cases it was necessary to incubate the cultures outdoors, where they were subject not only to fluctuating daylight but also to widely fluctuating temperatures (*15*). Only in this way, for example, was there obtained an abundance of well-developed sporodochia of the pitch canker *Fusarium* from pine.

THE FUSARIA ON ALBIZZIA

The demonstration, by Hepting (*5*) and Toole (*18*) in the United States and later by Campi (*2*) in Argentina, of a vascular fusarium wilt of the mimosa tree (*Albizzia julibrissin* Durazz.) and the description by Voronikhin (*21*) of what is probably the same disease in the

Soviet Union have made important the correct identification of the various species of *Fusarium*, some saprogenic, occurring on the mimosa tree.

NAMES PREVIOUSLY APPLIED TO THE WILT FUSARIUM

Culture studies and inoculations have demonstrated that the mimosa wilt *Fusarium* in the United States and Argentina is a form of *F. oxysporum*. In the following discussion the names that have correctly or mistakenly been applied to this fungus are taken up in order of their appearance in the literature.

Fusarium albizziae Voron. (*Nectria albizziae* Voron.)

In 1920, Voronikhin (*21*) described a disease of the mimosa tree, epidemic in the Soviet Union, that appears to agree in all respects with that later described in the United States by Hepting (*5*). Although Voronikhin demonstrated the presence of microconidia in the infected vessels and observed cushions of *Fusarium* macroconidia on the surface of shoots killed by the wilt, he apparently made no cultures of the fungus and no inoculations. Nor did Voronikhin present any evidence to show whether the *Fusarium* found on the bark was the same fungus as that seen in the vascular tissue or even whether it was pathogenic. Without proof of the connection between the surface *Fusarium*, which he described as *F. albizziae*, and his vascular pathogen, this name cannot be accepted as that of the causal agent of mimosa wilt. Voronikhin's descriptions indicate strongly that the bark *Fusarium* was not of the wilt (*oxysporum*) type.

Perithecia found on a branch of mimosa killed by wilt were described by Voronikhin as *Nectria albizziae*, on the basis of their association with the *Fusarium* also found fruiting on the bark. No proof of genetic relationship to the *Fusarium* was given, and therefore there is no certainty that the ascomycete was the same fungus. The perithecia were small and protruded in a group on a cushionlike stroma. No wilt Fusaria are known to produce perithecia, and thus additional doubt is attached to Voronikhin's conclusions about the connection of this fungus with the vascular parasite.

Fusarium perniciosum Hepting

Proof that the pathogen of the vascular wilt of mimosa is a *Fusarium* was first established by Hepting (*5*), who also noted the resemblance of the fungus he isolated to other vascular wilt Fusaria. Partly because of its selective pathogenicity for the mimosa tree and partly because of the short macroconidia in his cultures (mean length of 28μ for three-septate spores) Hepting named his fungus *F. perniciosum*, in keeping with the Wollenweber system of *Fusarium* classification. Hepting (*5*) and Toole (*18*) also mentioned clones differing widely from the common one in spore size and septation and in gross cultural appearance. These clones are now recognized merely as morphologic variants of the wilt fungus.

Fusarium oxysporum f. *perniciosum* (Hepting) Toole

This name was first suggested in a footnote by Toole (*18*) in 1941, but it was not proposed.

Fusarium oxysporum f. *perniciosum* (Hepting) Snyder

In 1943 Campi (*2*) published a paper in which the trinomial *F. oxysporum* f. *perniciosum* (Hepting) Snyder was used without Snyder's knowledge. Although Campi cited Toole's paper, either she did not see his footnote or she did not consider Toole's trinomial as valid under the rules, which state that the name of a taxonomic group is not validly published unless it is definitely accepted by the author who publishes it.

Campi's trinomial, however, also comes into question, since she did not definitely propose the combination but stated that *F. perniciosum*, according to the latest classification of Snyder, would be called *F. oxysporum* Schlecht. f. *perniciosum* (Hepting) Snyder.

NAME ACCEPTED FOR THE WILT FUSARIUM

In view of the confusion which exists in the nomenclature of the mimosa wilt pathogen, it seems desirable that a name validly published and still in accord with the trinomial system of *Fusarium* taxonomy (*13*, *14*, *16*) be indicated for acceptance.

It is proposed that the name *F. perniciosum* be changed, as suggested by Toole (*18*), in conformance with the *Fusarium* classification of Snyder and Hansen (*13*) as follows:

Fusarium oxysporum Schlecht. emend. Snyder and Hansen forma *perniciosum* (Hepting) Toole.

Syn. *F. perniciosum* Hepting (*5*).

F. oxysporum f. *perniciosum* (Hepting) Snyder (*2*).

A form of the species *F. oxysporum* pathogenic in the vascular tissues and causing a wilt in *Albizzia julibrissin* and by inoculation in *A. lebbeck* (L.) Benth., *A. lophantha* Benth., and *A. kalkora* (Roxb.) Prain (*18*). In southeastern United States, Argentina, and probably the Soviet Union.

ISOLATION OF FUSARIUM OXYSPORUM F. PERNICIOSUM

The mimosa wilt *Fusarium* may be obtained in culture by plating either the discolored vascular tissues of a diseased tree or the sporodochia which sometimes push out from the bark lenticels of a wilted tree when the environment is very humid. Sporodochia of some saprophytic Fusaria, however, occur even more commonly on the bark of dead or dying mimosa. Cultures made from sporodochia on the bark and from discolored wood beneath it have yielded identical clones of *F. oxysporum* f. *perniciosum* on several occasions, as shown by inoculation tests.

Hepting (*5*) found that several species of *Fusarium* may be recovered from mimosa bark and that at least one, which he recorded as *F. solani* var. *martii* (Appel and Wr.) Wr., could be isolated occasionally from diseased wood. This variety, synonymous with *F. solani* (Mart.) Appel and Wr. emend. Snyder and Hansen, was found to be unable to attack mimosa by itself (*5*); yet in nature it follows closely the wilt pathogen. This finding has recently been confirmed. Not only has *F. solani*, hidden in mixed cultures with *F. oxysporum* f. *perniciosum*,

been recovered from tissue platings of the discolored vascular region of wilted mimosa trees, but also the perfect stage, *Hypomyces solani* Reinke and Berth. emend. Snyder and Hansen, has been collected on the bark in association with sporodochia of *F. oxysporum* f. *perniciosum*. In fact, numbers of perithecia have been found in such cases clustered around sporodochia of the wilt *Fusarium*, an association which on the basis of appearances might well be considered to be that of a *Fusarium* and its *Nectria* or *Hypomyces* ascigerous stage. Only by making single-ascospore cultures from the perithecia and single-conidium cultures from the sporodochium in each case was the association detected. Perithecia of *Hypomyces* later developed in the single-ascospore cultures, especially in those incubated outdoors, demonstrating with certainty in these instances not only the identity of the fungus but also the fact that it was homothallic. Both heterothallic and homothallic strains of *H. solani* are known.

VARIABILITY IN FUSARIUM OXYSPORUM F. PERNICIOSUM

Although the use of natural clones is favored for the purpose of identification, it should be emphasized that natural clones may differ considerably among themselves (fig. 1). Some natural clones of the mimosa *Fusarium* produce abundant mycelium with but few slowly formed sporodochia; others form sporodochia rapidly and in abundance; still others are intermediate in appearance or differ in pigmentation from white to salmon to vinaceous shades or in their ability to develop sclerotia. Macroconidia may agree in size with those earlier described by Hepting for *F. perniciosum* (as in fig. 1, *C*), may be much larger (fig. 1, *D*) as in Hepting's Wisacky clone, or may otherwise reflect the variability in morphology displayed so commonly in the species *F. oxysporum*.

Selected natural clones, some of their variants, and their spores are shown for the mimosa wilt *Fusarium* in figure 1. The diverse clones shown here represent about 50 isolates made during the 1947 season, from different localities and States.

As an aid in recognizing the variability within this forma, the more common clones are described briefly as follows:

Clone A.—Commonest clone (fig. 1, *A*, last six tubes). Thallus raised; white, pink, or vinaceous shades; macroconidia rarely more than three-septate, produced abundantly in sporodochia and on surface of mycelium, salmon-colored in mass; microconidia abundant; no sclerotia, but often plectenchymic masses.

Clone B.—Hepting's Wisacky clone (fig. 1, *A*, middle three tubes). Thallus raised; white to vinaceous shades; macroconidia abundant, commonly four- or five-septate, produced in sporodochia and on surface of mycelium; microconidia abundant; blue-black sclerotia common.

Clone C.—Like clone A, but no sporodochia or plectenchymic masses (fig. 1, *A*, first three tubes, fig. 1, and *B*, fourth three tubes).

Clone D.—Pionnotal clone (fig. 1, *B*, first three tubes). No aerial mycelium; slimy spore mass over agar, mostly one- to three-septate macroconidia; some microconidia; color whitish to buff, pink or vinaceous; no sporodochia, plectenchymic masses, or sclerotia.

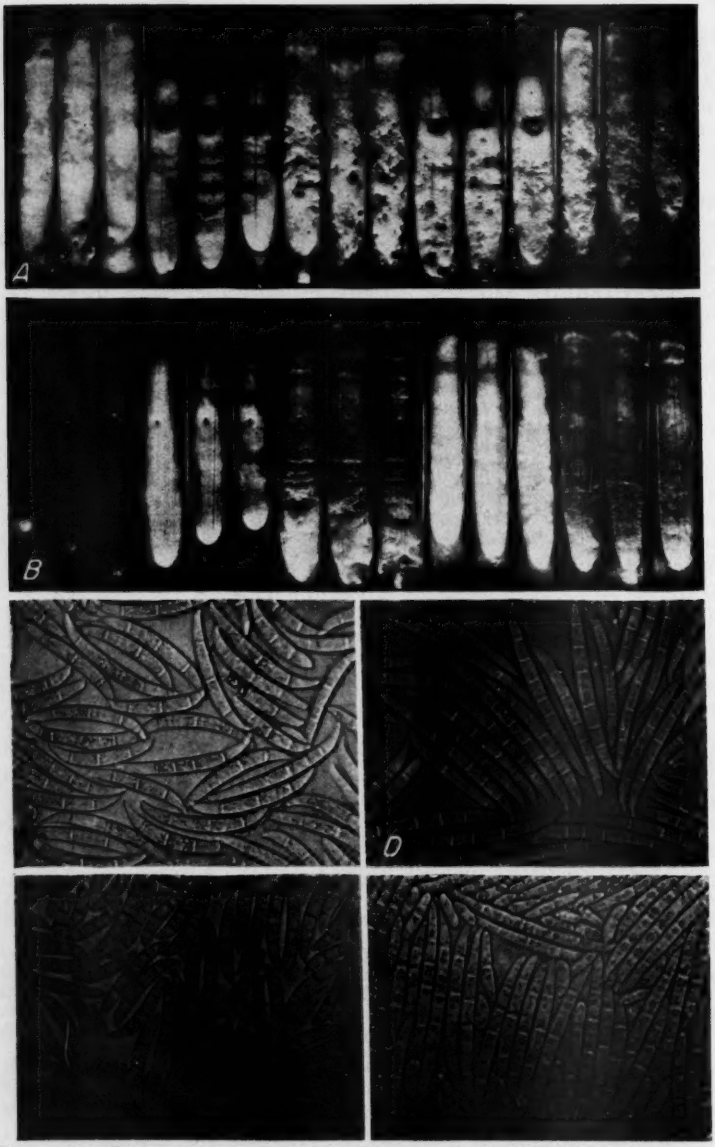

FIGURE 1.—*Fusarium oxysporum* f. *perniciosum*. *A* and *B*, Triplicate single-spore cultures of representative clones, made the same day on potato-dextrose agar: *A*, Identical with clones isolated directly from nature; *B*, laboratory mutants. *C*, Average-sized macroconidia from culture kept indoors. *D*, Long macroconidia from cultures made the same day from the same culture as those in *C* but kept outdoors. *E*, Very short macroconidia from a clone grown indoors. *F*, Long macroconidia from another clone grown indoors during the same period as those shown in *C*, *D*, and *E*. *C*–*F*, × 500.

FUNGI ASSOCIATED WITH MIMOSA WILT

The Hyphomycetes and Ascomycetes commonly found on the bark of wilted mimosa in this investigation may be characterized briefly as follows:

Fusarium oxysporum f. *perniciosum*

Flesh-colored sporodochia, composed mostly of macroconidia, protruding from lenticels. In culture: pigmentation and cultural pattern various (see p. 370). Microconidia; macroconidia three- to five-septate, usually widest in the upper third; terminal and intercalary chlamydospores in the mycelium and spores.

Cultures of a form of *F. oxysporum* isolated from discolored xylem of a dying tree of *Albizzia procera* (Roxb.) Benth. in Puerto Rico failed to induce wilt in *A. julibrissin* in a small-scale test.

Fusarium solani (*Hypomyces solani*)

Cream, green, or blue (sometimes turning brown with age) sporodochia of macroconidia on bark or protruding from lenticels. In culture: cream, green, or blue pigmentation associated with spore masses, but mycelium usually not pigmented. Microconidia; terminal and intercalary chlamydospores in the mycelium and spores; macroconidia three- to five-septate or more, curved-cylindrical, apical cells usually blunt.

Ascigerous stage a *Hypomyces;* perithecia red, usually in groups around a sporodochium; ascospores hyaline, one-septate, usually tan-colored in mass.

Fusarium decemcellulare Brick (*Calonectria rigidiuscula* (Berk. and Br.) Sacc.)

Cream-colored spore masses inconspicuous, small, on bark. In culture: pigmentation next to agar usually carmine red, aerial mycelium white. Microconidia in chains, conspicuous; chlamydospores lacking; macroconidia in creamy masses, very large, 5- to 11-septate, curved-cylindrical as in *F. solani.*

The ascigerous stage was not observed on bark. Perithecia yellow to brown; ascospores hyaline, curved, usually three-septate, tan-colored in mass.

Fusarium episphaeria Snyder and Hansen (*Nectria episphaeria* Tode ex Fr.)

Conidial stage on bark inconspicuous or a flesh-colored slime. In culture: very slow-growing colonies, salmon to pink or orange-colored, consisting mostly of prostrate mycelium covered with a conidial slime, aerial mycelium usually absent. Microconidia usually absent, chlamydospores present or absent, macroconidia long, slender, one- to seven-septate.

Ascigerous stage in *Nectria.* Perithecia quite small, red, usually in groups on bark invaded by sphaeriaceous fungi; ascospores hyaline, one-septate, usually tan-colored in mass. (Not to be confused with another *Nectria* species which has similar perithecia, but an imperfect stage not in *Fusarium,* possibly in *Tubercularia.*)

Voronikhin's *F. albizziae* probably is a member of this species, and possibly his *Nectria albizziae* is also.

Fusarium lateritium Nees ex Fr. (*Gibberella lateritia* Snyder and Hansen)

Bright-pink to orange sporodochia, protruding from lenticels, consisting of macroconidia. In culture: pink, flesh, or orange sporodochia; mycelium white, pink, or variously colored, sometimes carmine red or white where in contact with the agar, colonies usually slower growing than those of *F. oxysporum* or *F. solani,* but faster growing than those of *F. episphaeria.* Microconidia and chlamydospores usually lacking. Macroconidia resembling those in *F. oxysporum* but usually longer, often with a tendency for the terminal cell to be hooked; three- to five-septate.

Ascigerous stage suspected on mimosa but not observed. Perithecia blue black, in groups; ascospores hyaline, curved, mostly three-septate, usually tan-colored in mass.

Fusarium roseum Lk. ex Fr. (*Gibberella rosea* Snyder and Hansen)

Bright-pink to flesh-colored sporodochia, often small but otherwise resembling those of *F. lateritium.* In culture: mycelium white, yellow, or red, very fast growing but often slow to produce conidia. Microconidia usually lacking; chlamydospores present or absent; macroconidia in flesh- to tan-colored sporodochia, apical cell not hooked.

Ascigerous stage not observed on mimosa. Perithecia blue black; ascospores hyaline, curved, usually three-septate, tan-colored in mass.

Tuberculariа vulgaris Tode ex Fr. (*Nectria cinnabarina* Tode ex Fr.)

Conspicuous pink sporodochial cushions, protruding from lenticels, composed of microconidia. In culture: moderately scant white mycelium, producing pink to flesh-colored sporodochia of the *Tuberculariа* stage in outdoor culture.

Ascigerous stage grouped around the sporodochia. Perithecia red, resembling those of *Hypomyces solani*; ascospores hyaline, one-septate. Observed at Washington, D. C., by Fowler and Stevenson (*3*).

Thyronectria austro-americana (Speg.) Seeler

Perithecia in clusters, light to dark brown; ascospores hyaline to pale straw-colored, three- to six-septate, muriform, often budding in the ascus and producing numerous ascoconidia.

Conidia of the *Gyrostroma* stage produced in pycnidia in the stroma, hyaline, nonseptate, orange-colored in mass.

Eutypa heteracantha Sacc.

Black perithecia grouped in a stroma, beaks protruding; ascospores hyaline, nonseptate. Fruiting commonly on dead bark of mimosa and frequently providing the substrate upon which *Nectria episphaeria* and *Nectria* sp. were found to develop.

THE SUMAC WILT FUSARIUM

During the summer of 1946 wilting and dying of staghorn sumac (*Rhus typhina* L.) were observed by G. H. Hepting along the Blue Ridge Parkway, near Waynesboro, Va. Examination of diseased plants disclosed that the disease was a vascular wilt, and cultures from the discolored xylem yielded a form of *Fusarium oxysporum* [6] which has been shown to be pathogenic (*20*). Observations during the 1947 season were concerned principally with the extent of the infection in Virginia, inoculation tests, and taxonomic studies.

TAXONOMY AND NOMENCLATURE

Isolations of the causal *Fusarium* were made by tissue platings from the xylem of root, crown, trunk, branch, petiole, and base of the fruiting cluster of wilted plants. In each case, single-spore transfers were made directly from the sporulation on the bits of tissue. Also, numerous single-spore isolations were made directly from sporodochia found abundantly on the trunks and sometimes on the lower branches of wilted trees, especially where the sumac was located in moist or densely vegetated stands. In repeated isolations identical clones of a *Fusarium* were obtained from the sporodochia on the bark and the discolored xylem beneath it. These observations, together with inoculation data (*20*), showed that this wilt *Fusarium* can produce sporodochia on the bark, as in the case of the mimosa wilt fungus. *F. solani* was also isolated from the bark of diseased sumac, and its ascigerous stage, *Hypomyces solani*, was observed and identified with it culturally.

The sumac wilt *Fusarium* was typical of *F. oxysporum* in that, on potato-dextrose agar, it produced a white mycelial colony which later developed typical sporodochia. Microconidia developed abundantly and in false heads, intercalary and terminal chlamydospores were common in the mycelium and in old conidia, and the macroconidia were characteristic of the species (fig. 2).

[6] Original isolations were made by R. W. Davidson, of the Division of Forest Pathology, United States Department of Agriculture, and original determination as *F. oxysporum* was made by H. N. Hansen and W. C. Snyder, of the Division of Plant Pathology, University of California.

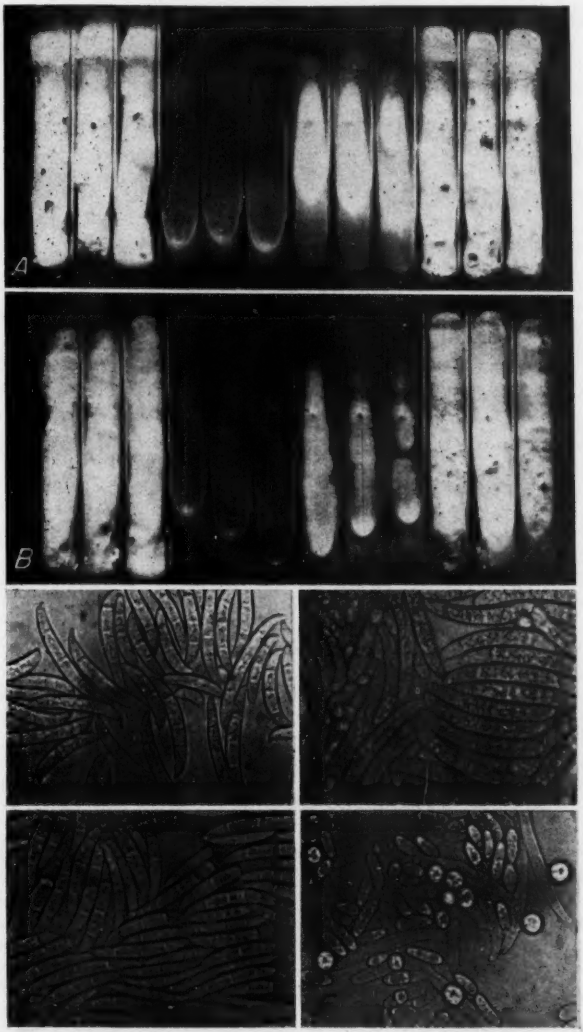

FIGURE 2.—*A*, Triplicate cultures of representative clones of *Fusarium oxysporum* f. *rhois* (first and last three tubes, natural clones; third three, going into the pionnotal clone; and second three, pionnotal mutants). *B*, Clones of *F. oxysporum* f. *perniciosum* arranged below those in *A* to show their similarity to clones of *F. oxysporum* f. *rhois*, when grown from single spores sown the same day on the same batch of medium. *C–F*, *F. oxysporum* f. *rhois*: *C*, Small macroconidia produced indoors; *D*, large macroconidia and microconidia of the clone shown in *C* but produced outdoors; *E*, average-sized macroconidia; *F*, microconidia and chlamydospores mixed with a few macrospores. *C–F*, × 500.

Single-spore clones of the sumac fungus have been found to belong to the species *F. oxysporum*, and these proved to be pathogenic to sumac. Since cross-inoculations with *F. oxysporum* f. *perniciosum*, the only other known vascular *Fusarium* pathogen of a tree, have been negative, the sumac pathogen is described here in conformity with the classification and nomenclature of Snyder and Hansen (*13*) as a new forma of this species. The following trinomial is proposed:

Fusarium oxysporum Schlecht. emend. Snyder and Hansen forma *rhois* Snyder and Hepting form. nov.

Parasitic in the vascular system and causing a wilt disease of *Rhus typhina* in the Blue Ridge Mountains of Virginia.[7]

VARIABILITY IN FUSARIUM OXYSPORUM F. RHOIS

The occurrence in nature of only one clone of this vascular pathogen is in sharp contrast to the mimosa wilt fungus, for which numerous natural clones have been established. The reason for this difference may be the fact that the sumac disease so far is known in only one small area, a few miles long. It seems possible that only one natural clone has established itself or has originated in this area, whichever the case may be. The mimosa wilt, on the other hand, is widespread throughout many States in the Southeast (*19*) and in a great variety of soil and climatic environments. Such a situation would be expected to result in time in the occurrence of geographical or ecological natural clones best suited to these different environments.

Although the isolates from nature so far have belonged to one clone, this clone, when cultivated in the laboratory in pure culture on potato-dextrose agar, has proved to be very unstable. Single-spore cultures of the natural clone, which is the typical sporodochial clone of *F. oxysporum*, have repeatedly yielded several mutants each succeeding time that they were single-spored. The most common mutant which continually arises from the sporodochial natural clone is the pionnotal one (fig. 2, *A*, second three tubes), and this may be considered as an expression of the Hansen dual phenomenon (*4*).

SIMILARITY BETWEEN THE SUMAC AND MIMOSA PATHOGENS

Although *F. oxysporum* f. *perniciosum* and *F. oxysporum* f. *rhois* are distinct biologically in that neither is pathogenic to the host of the other, their similarity in culture is very close. The natural clone of the sumac fungus may be matched in appearance closely with certain natural clones of the mimosa fungus. Furthermore, some of the mutants obtained from the mimosa fungus are almost identical in appearance with those from the sumac *Fusarium* (fig. 2, *A* and *B*), and no way has been found to tell them apart in their microscopic characters. The natural clone corresponds in appearance to clone *B* of *F. oxysporum* f. *perniciosum*, and it is known to throw mutants that agree with clones *C* and *D* of that fungus. These observations

[7] The writers are indebted to E. K. Cash, Division of Mycology and Disease Survey, Bureau of Plant Industry, Soils, and Agricultural Engineering, for her suggestion on the derivation of the forma name from *Rhus*.

further emphasize the basis for grouping them in the same species, yet keeping them separate on the basis of biological habit.

THE PINE PITCH CANKER FUSARIUM

The identity and characteristics of the *Fusarium* that causes the pitch canker disease of pine (*8*) are of particular interest (1) because this is the first disease of pines, other than seedling diseases, that has been ascribed to a *Fusarium* and (2) because of the value that this organism may have in prolonging gum flow from chipped pines in the naval stores industry (*6*). Whether the primary consideration may be the control of the pathogen responsible for pitch canker or the cultivation of, distribution of, and inoculation with a *Fusarium* beneficial in the production of turpentine and rosin, either the control or utilization of this organism depends upon an understanding of its identity and its behavior both in nature and in laboratory culture.

NATURAL DISTRIBUTION

Up to the present time the pitch canker fungus has been isolated from several species of southern pines in parts of Virginia, North Carolina, Tennessee, South Carolina, Georgia, Alabama, and Florida. Many natural cankers have been examined, especially in the North Carolina range of the disease, without finding fruiting of the *Fusarium*. This lack of fruiting of any kind has increased the difficulties in determining the kind of *Fusarium* involved. In old, inactive cankers the fungus is usually dead, and there is little promise of finding the fruiting stages on such material.

The only sporulation seen in the field on pines has been where the bark-covered wood of an active canker was artificially exposed or on the chipped surfaces of pines tapped for gum and artificially inoculated with the fungus. In these cases the *Fusarium* produces a light, white, patchy growth of mycelium on the wood itself. Later these patches often become pink and produce moderate amounts of conidia, most of which are microconidia but a few of which are macroconidia. This behavior, together with the fact that normal sporulation on undisturbed natural cankers has not been seen, suggests that the pine is not the only host of the *Fusarium*, but that under certain conditions the fungus has been able to establish itself on pine from inoculum perhaps produced on a very different host. Natural infection and distribution may take place through the aid of insects often found in connection with small new infections and observed to emerge from old cankers.

THE PINE FUSARIUM IN CULTURE

Since natural sporulation has been wanting, isolation of the pitch canker pathogen has depended upon the culture of bits of tissue taken from the wood and inner bark of active cankers (*8*). Single-spore isolations made directly from such tissue platings have given pure cultures of several natural clones.

During the 1947 season about 60 single-spore isolates of the *Fusarium* from pitch cankers in the aforementioned States and from 4 species of pine (*Pinus virginiana* Mill., *P. echinata* Mill., *P. caribaea* Mor., and *P. palustris* Mill.) were assembled. Most of these isolates

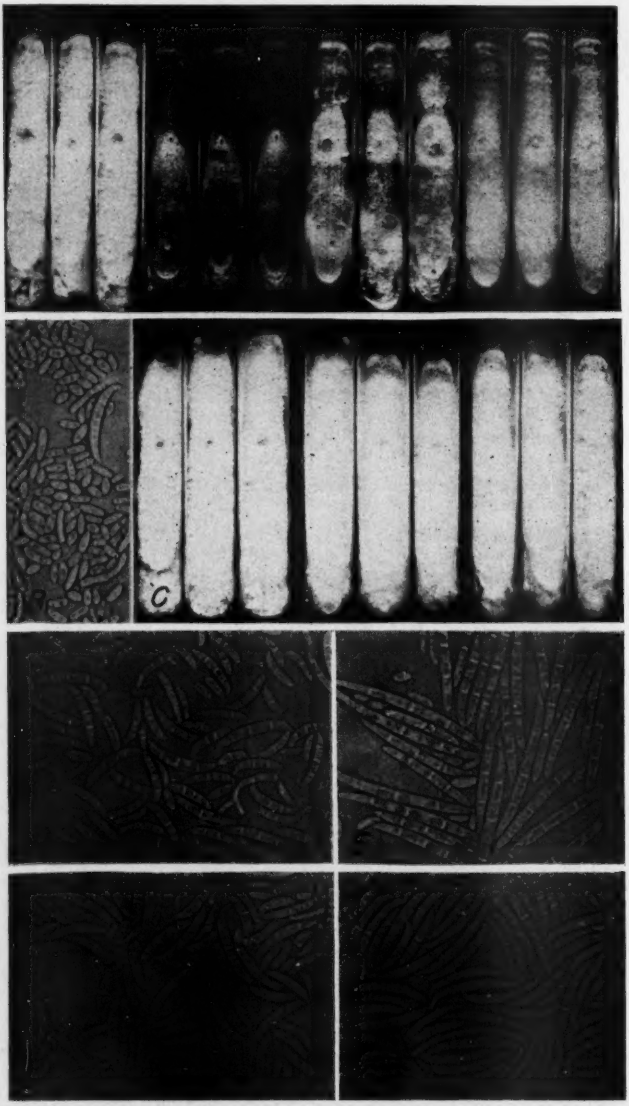

FIGURE 3.—*Fusarium lateritium* f. *pini*. *A*, Triplicate cultures of representative clones. *B*, Microconidia mixed with a few macrospores. *C*, Triplicate cultures of identical natural clones (type C) from *Pinus virginiana*, *P. palustris*, and *P. caribaea*, respectively. *D–G*, Macroconidia of different clones, all grown during the same period from single-spore cultures, showing great variation in size and shape. *B* and *D–G*, × 500.

represent natural clones, but a few were laboratory mutants.[8] These cultures provided the basis for the observations made on taxonomy and pathogenicity.

All natural clones in culture in the laboratory were characterized by a white fluffy mycelium which, in some clones, tended with age, to become pigmented in shades of blue, gray blue, green blue, and purple, especially in diffuse daylight. Many clones developed a purple pigment in the mycelium lying in contact with the potato-dextrose-agar slant. Some clones produced dark-bluish sclerotia. Many other natural clones differed in these and in other characters such as colony texture, configuration, zonation, and rate of growth (fig. 3, *A*).

Microscopically the laboratory-grown cultures were unsuited for taxonomic study, because macroconidia were almost entirely lacking even though the cultures were grown in light. In this absence of macroconidia the pine *Fusarium* could have fallen into the species *F. moniliforme*, into *F. oxysporum* if certain swellings in the mycelium were interpreted as chlamydospores, or into *F. lateritium*.

A good supply of normal macroconidia in sporodochia were produced on potato-dextrose cultures kept outdoors in the fall and subject to wide fluctuations in diffuse light and in temperature. Companion cultures of the same clones made at the same time on the same batch of medium but incubated in the laboratory near the window, produced no sporodochia.

The sporodochia of all natural clones grown outdoors were orange-colored, and the masses of macroconidia were often in sufficient abundance to give the entire slant an orange cast. The masses of macroconidia began to appear about a month after the cultures were placed outdoors, but they reached their greatest development in about 6 weeks.

Macroconidia were three- to five-septate, with a tendency toward the formation of a slight hook of the apical cell, characteristic of the small-spored types in *F. lateritium*. The apical-cell hook, the preponderance of macroconidia in optimum culture, and the absence of both chlamydospores and chain-formed microconidia place the fungus in *F. lateritium*. The fungus was first published under this name in 1947 (7).

The variation in cultural forms and in spore characteristics is evident in figure 3. Mutations from natural clones were occasionally obtained in the laboratory through single-spore transfer from single-spore cultures. The commonest mutant, recovered from several clones, was a pionnotal variant. Such cultures produced little aerial mycelium and a slime of spores on the surface of the agar and usually were deeper purple than the parent. The macroconidia of the pionnotal clones were generally much longer than those of the parent (fig. 3). Other mutants differed in the amount of pigmentation, amount and character of mycelium, and rate of growth.

[8] Cultures and fresh canker material from Georgia and Florida were supplied by R. P. True and from Alabama by R. M. Lindgren, both of the Division of Forest Pathology.

TAXONOMY OF THE PINE FUSARIUM

After a study of cultures from sporodochial natural clones had established the fact that the pine *Fusarium* was *F. lateritium*, it was necessary to determine whether only clones of this species from pine would cause pitch canker and gum flow. To test this point, fresh isolates of a *F. lateritium* from *Albizzia* were used to make parallel inoculations in Virginia pine in conjunction with isolates of the pine form. Cankers and gum flow were obtained with isolates from pine only.

In view of the distinctive pathogenicity on pine of the isolates obtained from pitch cankers and the unique economic potentialities of this fungus in the naval stores industry, it is proposed to designate the pine *Fusarium* as a new form as follows:

F. lateritium Nees emend. Snyder and Hansen forma pini Hepting form. nov.

Pathogenic on the trunk, leader, and branches of pine (*Pinus virginiana*, *P. echinata*, *P. caribaea*, and *P. palustris*), causing thereon cankers characterized by heavy gum flow. Also prolongs gum flow from these hosts when applied to fresh wounds on them. Natural habitat, southeastern United States.

Clones grown on potato-dextrose agar in diffuse daylight include the following and various intermediate stages between them:

Clone A.—Common clone (fig. 3, *A*, first three tubes). Fast-growing, raised mycelial thallus, white through vinaceous shades, producing abundant microconidia in false heads indoors and abundant three- to five-septate macroconidia on sporodochia outdoors.

Clone B.—Sclerotial clone (fig. 3, *A*, third three tubes). Similar to *A*, but producing abundant sclerotia or blue-black plectenchyma.

Clone C.—Similar to clones *A* and *B*, but producing neither sporodochia nor sclerotia (fig. 3, *A*, fourth three tubes).

Clone D.—Pionnotal clone (fig. 3, *A*, second three tubes). No aerial mycelium. Macroconidia and microconidia in a slime over the agar. Whitish to vinaceous shades.

Clone E.—Very slow growing, bright purplish blue, producing mostly microconidia even outdoors, with semiraised mycelium.

The only other *Fusarium* isolated from pine wounds in this study was *F. roseum*. Both red and yellow-brown natural clones of this species were recovered. They failed to produce lesions when inoculated on *Pinus virginiana*. The *Gibberella rosea* stage was obtained in single-conidium cultures incubated outdoors during October and November.

DISCUSSION

In isolating clones of *Fusarium* species from nature, it is desirable that single-spore or single-hyphal-tip techniques be used either directly from the host or from the first tissue isolates. One of the most important reasons for this requirement is the tendency of all three pathogenic Fusaria herein described to live in close association with other Fusaria, notably *F. solani*, not only on the same host, but sometimes even in the same bark pustule or in the same vascular tissue. Natural clones were maintained through successive culturing by single-sporing all transfers and retaining those that were identical with the original clone.

The importance in identification of working with a large number of cultures transferred the same day on the same batch of medium was clearly shown by the varying effects of different environments on the appearance of cultures of parallel series during the 5 months in which these studies were carried on. For example, cultures of *F. lateritium* f. *pini* kept indoors in diffuse light produced abundant microconidia and practically no macroconidia, and such cultures would probably never have been identified as that species. Parallel cultures stored outdoors produced excellent sporodochia, abundant macroconidia, and almost no microconidia and were readily placed under *F. lateritium*. Cultures of *F. oxysporum* f. *perniciosum* kept indoors produced for the most part relatively short macroconidia, although some produced long macroconidia; whereas outdoor cultures produced macroconidia that were of average length for *F. oxysporum* generally. Thus some special handling, approaching more natural conditions (*17*), is often required to get good development of the structures used in identification, and the parallel study of many isolates is necessary to acquaint an investigator with the variations that may occur in a given species and forma.

The pine canker and mimosa wilt fungi illustrate in a striking way the multiplicity of morphologic clones of certain Fusaria in nature. These results, together with those of Borlaug (*1*) on flax wilt, Nelson, Coons, and Cochran (*11*) on celery wilt, and Snyder (*12*) on pea wilt, fail to substantiate Miller's (*10*) concept that usually there is only one "wild type," or natural clone, for a given *Fusarium*. The work just cited and the current studies on *F. oxysporum* f. *perniciosum* and *F. lateritium* f. *pini* show for several Fusaria wide variability in isolates made on the same medium directly from nature. Comparisons made by some workers of isolates of a given *Fusarium* obtained from a variety of sources over a wide range of years are not accepted by Miller as valid comparisons of natural clones, since some mutations undoubtedly took place in the meantime. In the case of Miller's muskmelon *Fusarium* in Canada (*9*) and of the sumac *Fusarium*, the occurrence of one predominating natural clone seems to be the rule. However, the variation in natural clones of the flax wilt, pea wilt, celery wilt, pine canker, and mimosa wilt Fusaria, in addition to the experience of the writers with other Fusaria, indicates that Miller's assertion (*10*) that one "wild type" predominates and that variation in types isolated directly from nature "must be relatively infrequent" should not be applied to Fusaria in general.

The morphological variability shown within the pathogenic formae of the mimosa wilt and pine canker Fusaria follows the pattern of other Fusaria (*1*, *9*, *10*, *11*, *12*, *13*, *14*, *16*, *22*) and poses important questions concerning the identification of such pathogens, procedures for the maintenance of stock cultures, selection of the clone for deposition in type-culture collections, and choice of clones for use in projects aimed at nomenclature, disease resistance, and control. Certainly an intelligent approach to the problems presented by these diseases requires recognition of the phenomenon of morphologic and physiologic variability demonstrated to occur in nature.

SUMMARY

Clones of Fusaria pathogenic to species of *Albizzia*, *Pinus*, and *Rhus*, isolated in pure culture from trees in numerous locations, provided the bases for the comparative studies on the cultural characteristics, taxonomy, and nomenclature of these fungi. Single-spore or single-hyphal-tip cultures made directly from the host or from fresh-tissue cultures were used throughout the work, both in the isolation of natural clones and in subsequent subculturing.

Although one or more clones of each of the three pathogens were shown to be variable in culture through mutation, two of the pathogens—those of *Albizzia* and of *Pinus*—were found to exist in nature in a highly variable state, each consisting of numerous distinct, morphologic clones.

The trinomial *Fusarium oxysporum* f. *perniciosum* (Hepting) Toole is accepted for the mimosa wilt pathogen; *F. oxysporum* f. *rhois* Snyder and Hepting is proposed for the sumac wilt pathogen; and *F. lateritium* f. *pini* Hepting is proposed for the pine pitch canker *Fusarium*.

Procedures applicable to pure-culture isolation, the maintenance of original clones, and the induction of optimum sporulation are outlined for each pathogen, and brief descriptions of clones are provided as a guide to their identification. Sporodochia of the pitch canker *Fusarium* were obtained only in outdoor culture under conditions of fluctuating light and temperature.

Consideration is given also to the saprophytes commonly associated with the above-named pathogens in diseased trees.

Some sporodochia on the bark of diseased mimosa and sumac yielded pure cultures of the pathogens, whereas others proved to be those of saprophytic Fusaria. No sporulation of the pitch canker *Fusarium* was observed on natural cankers in the field, but some occurred on chipped faces of pines artificially inoculated.

LITERATURE CITED

(1) Borlaug, N. E.
1945. variation and variability of fusarium lini. Minn. Agr. Expt. Sta. Tech. Bul. 168, 40 pp., illus.

(2) Campi, M. D.
1943. el marchitamiento de la acacia de constantinopla ("albizzia julibrissin") y su relación con la presencia de "fusarium oxysporum" schl. f. "perniciosum" (hepting) snyder. Lilloa 9: [457]–460, illus.

(3) Fowler, M. E., and Stevenson, J. A.
1944. a canker and some decay fungi on mimosa. (Phytopath. note) Phytopathology 34: 985–987, illus.

(4) Hansen, H. N.
1938. the dual phenomenon in imperfect fungi. Mycologia 30: 442–455, illus.

(5) Hepting, G. H.
1939. a vascular wilt of the mimosa tree (albizzia julibrissin). U. S. Dept. Agr. Cir. 535, 11 pp., illus.

(6) ———
1947. stimulation of oleoresin flow in pines by a fungus. Science 105: 209, illus.

(7) ———
1947. stimulation of gum flow by a fungus. Forest Farmer 6 (6): 2.

(8) ——— and Roth, E. R.
1946. pitch canker, a new disease of some southern pines. Jour. Forestry 44: 742–744, illus.

(9) Miller, J. J.
1945. studies on the fusarium of muskmelon wilt. I. pathogenic and cultural studies with particular reference to the cause and nature of variation in the causal organism. Canad. Jour. Res., Sect. C, 23: 16–43, illus.

(10) ———
1946. cultural and taxonomic studies on certain fusaria. I. mutation in culture. Canad. Jour. Res., Sect. C, 24: 188–212, illus.

(11) Nelson, R., Coons, G. H., and Cochran, L. C.
1937. the fusarium yellows disease of celery . . . Mich. Agr. Expt. Sta. Tech. Bul. 155, 74 pp., illus.

(12) Snyder, W. C.
1933. variability in the pea-wilt organism, fusarium orthoceras var. pisi. Jour. Agr. Res. 47: 65–88, illus.

(13) ——— and Hansen, H. N.
1940. the species concept in fusarium. Amer. Jour. Bot. 27: 64–67.

(14) ——— and Hansen, H. N.
1941. the species concept in fusarium with reference to section martiella. Amer. Jour. Bot. 28: 738–742.

(15) ——— and Hansen, H. N.
1941. the effect of light on taxonomic characters in fusarium. Mycologia 33: 580–591, illus.

(16) ——— and Hansen, H. N.
1945. the species concept in fusarium with reference to discolor and other sections. Amer. Jour. Bot. 32: 657–666.

(17) ——— and Hansen, H. N.
1947. advantages of natural media and environments in the culture of fungi. (Phytopath. note) Phytopathology 37: 420–421.

(18) Toole, E. R.
1941. fusarium wilt of the mimosa tree (albizzia julibrissin). Phytopathology 31: 599–616, illus.

(19) ———
1948. distribution of mimosa wilt in 1947. U. S. Bur. Plant Indus., Soils, and Agr. Engin., Plant Dis. Rptr. 32: 67, illus. [Processed]

(20) ——— Snyder, W. C., and Hepting, G. H.
1948. a new fusarium wilt of sumac. (Abstract) Phytopathology 38: 572.

(21) VORONIKHIN [WORONICHIN], N. N.
1920. FUSARIUM ALBIZZIAE N. SP., CAUSE PROBABLE DU DÉPÉRISSEMENT D'ALBIZZIA JULIBRISSIN BOISS. DANS L'ARRONDISSEMENT DE BATUM (communication préliminaire). Vest. Tiflis. Bot. Sada (Monit. Jard. Bot. de Tiflis) 48: [15]–36, illus. [In Russian. French summary, pp. 34–36.]
(22) WOLLENWEBER, H. W., and REINKING, O. A.
1935. DIE FUSARIEN: IHRE BESCHREIBUNG, SCHADWIRKUNG UND BEKÄMPFUNG. 355 pp., illus. Berlin.

PROBLEMS RELATING TO THE REMOVAL OF DDT SPRAY RESIDUE FROM APPLES[1]

By KENNETH C. WALKER

Assistant chemist, Washington Agricultural Experiment Station

I. EFFECT OF WASHING ON REMOVAL OF DDT RESIDUE

The increased use from 1931 to 1946 of acid lead arsenate-petroleum oil mixtures, of cryolite-petroleum oil mixtures, or both for the control of the codling moth in the orchards of north-central Washington necessitated the development and adoption of uniform chemical washing practices for the removal of spray residues. Generally, a lead arsenate-oil or cryolite-oil spray program consisted of five to eight cover sprays, at least three of which contained oil at the rate of 2 quarts or more per 100 gallons of spray. To remove the spray residue from fruit sprayed with these mixtures, it has been necessary to use a two-unit (tandem) type washer.[2 3] Most of the commercial warehouses in the north-central Washington fruit district have employed a fruit washing system similar to the one outlined below.

First unit—40 to 60 pounds of sodium silicate per 100 gallons of water at 90° to 100° F.

Warm water rinse.

Second unit—1.5 percent hydrochloric acid at 90° to 100° F.

Copious fresh water rinse.

The results obtained in codling moth control from the use of DDT in the experimental spray plots conducted by various investigators of State and Federal agencies forecast the widespread use of this material in commercial orchards in 1947. Experimental washing studies were therefore undertaken to determine the effectiveness of commercial warehouse washing practices when employed for the removal of DDT from apples.[4 5]

---

[1] Received for publication June 16, 1948. Published as Scientific Paper No. 773, College of Agriculture and Agricultural Experiment Stations, Institute of Agricultural Sciences, State College of Washington.

[2] SMITH, E., RYALL, A. L., GROSS, C. R., and others. THE REMOVAL OF LEAD, ARSENIC AND FLOURINE RESIDUES FROM APPLES. Wash. State Hort. Assoc. Proc. (1933) 29: 86–96. [1934.]

[3] OVERLEY, F. L., OVERHOLSER, E. L., ST. JOHN, J. L., and GROVES, K. FURTHER EXPERIMENTS ON SPRAY RESIDUE REMOVAL. Wash State Hort. Assoc. Proc. (1934) 30: 77–82. [1935.]

[4] MANALO, G. D., HUTSON, R., MILLER, E. J., and BENNE, E. J. REMOVING DDT SPRAY RESIDUE FROM APPLES. Food Packer 27: 64, 66, 68, illus. 1946.

[5] ROBINSON, R. H. DDT RESIDUE PROBLEMS ON APPLES AND PEARS. Oreg. Agr. Expt. Sta. Cir. Inform. 412, 4 pp., 1947. [Processed.]

Fruit from experimental plots were selected for residue sampling. All samples were taken by sampling at random two or three fruits from each picked box until one-half to one full box was collected. All samples were divided into eight subsamples and two of the subsamples were analyzed for original DDT residue. The remaining samples were washed in a single-unit Cutler washer with the following characteristics:

Time of one revolution	3.3 seconds
Length of step-over (carrier)	4 inches
Length of tank	33 inches
Time in machine	26.8 seconds

Two samples were washed in 1.5 percent hydrochloric acid at 100° F.; two samples were washed in 60 pounds of sodium silicate per 100 gallons of water at 100°; and then, in an effort to duplicate the tandem wash used in commercial practices, two samples were washed in the sodium silicate at 100° and rerun through the same machine after it had been cleaned and refilled with 1.5 percent hydrochloric acid at 100°.

DDT residue was determined by treating approximately 10 fruits in the laboratory with a known volume of toluene in a tumbling machine for a 5-minute period. An aliquot of the toluene was treated with 50 milliliters of liquid ammonia and enough metallic sodium to form a permanent blue color in the ammonia layer. After standing one-half hour, the excess sodium was destroyed with methyl alcohol and the samples evaporated to dryness on a hot plate. The sample was picked up with 25 milliliters of water, 5 milliliters of nitric acid was added, and the chlorine was determined by the Volhard method.

Forty-two samples with an original residue load ranging from 5.0 to 22.3 parts per million were used in each of the washing trials. Replicate samples were used in determining the original residue load and in each of the washing trials.

The percentage of samples with a DDT residue equal to or greater than the present permissible tolerance of 7 parts per million and the percentage of samples showing reduced amounts of residue due to washing are presented in table 1. Slightly less than one-half (47.6 percent) of the samples that received no treatment carried a DDT residue of more than 7 parts per million. The hydrochloric acid treatment decreased the percentage of samples with a DDT residue of more than 7 parts per million to 35.7 percent, or an increase of 25 percent over the samples that received no treatment. The sodium silicate treatment and the tandem treatment decreased the percentage of samples with a DDT residue of more than 7 parts per million to 28.6 percent, or an increase of 39.9 percent over the samples that received no treatment. For DDT residue removal, the one-bath sodium silicate treatment was more efficient than the one-bath hydrochloric acid treatment and as efficient as the tandem treatment.

Significant amounts (at the 5-percent level) of DDT residue were removed from 78.6 percent of the hydrochloric acid washed samples, 88.1 percent of the sodium silicate washed samples, and 90.5 percent of the tandem washed samples. Although the washing treatments removed significant amounts of residue, none of them removed sufficient amounts of DDT from all samples to make the methods practicable for commercial use.

TABLE 1.—*Percentage of samples showing DDT residue above 7 p. p. m. after washing and percentage of samples showing reduced amounts of residue*

Treatment	Samples above 7 p. p. m.	Effectiveness of treatment
	Percent	*Percent*
No washing	47.6	0
Hydrochloric acid	35.7	25.0
Sodium silicate	28.6	39.9
Tandem	28.6	39.9

For the purpose of comparison, the samples were divided into three classes: (1) Those samples with an original DDT residue of less than 7 parts per million; (2) those samples with an original DDT residue of more than 7 parts per million; and (3) all samples regardless of the amount of the original DDT residue. The mean amount of DDT residue removed by the treatments in each of the above classes in presented in table 2.

TABLE 2.—*Mean amount of DDT residue removed*

Treatment	Class 1	Class 2	Class 3
	Original residue less than 7 p. p. m.	Original residue more than 7 p. p. m.	All samples
	P. p. m.	*P. p. m.*	*P. p. m.*
Hydrochloric acid	1.5	1.7	1.6
Sodium silicate	1.9	3.1**	2.5**
Tandem	2.2	3.3**	2.7**

**Significant at the 5-percent level.

The sodium silicate wash and the tandem wash removed significantly greater amounts of DDT residue in class 2 and class 3 samples than the hydrochloric acid wash. In the class 1 samples, all treatments removed approximately the same amount of DDT residue. In all three classes, the amount of DDT residue removed by the sodium silicate wash was very nearly the same as that removed by the tandem wash, and there was no significant difference between these two treatments. The sodium silicate wash was more efficient in residue removal than the hydrochloric acid wash and was as efficient as the tandem wash.

Regression analysis comparing the original DDT residue present on the unwashed samples and the amount removed by washing was made of each treatment and no significant relationship was found. Percentage residue removal was calculated for each sample, for each treatment, and was found to vary from a negative 32.8 percent to a positive 69.5 percent. No correlation could be established between the percentage of DDT residue removed and the amount originally present. The average removal by the hydrochloric acid treatment was found to be 21.3 percent, by the sodium silicate treatment, 29.5 percent, and by the tandem wash treatment, 35.6 percent.

II. EFFECT OF CERTAIN STICKERS AND SPREADERS ON DDT RESIDUE AND OF OVERHEAD SPRINKLING ON REMOVAL OF DDT RESIDUE

During the 1947 growing season in north-central Washington there was an increased use of orchard sprinkling irrigation systems and, as expected, a change of spray programs from lead arsenate-oil mixtures and cryolite-oil mixtures to DDT dispersed in water. Studies were accordingly made to determine the effect of certain stickers and spreaders on the DDT residue and the effect of overhead sprinklers on the removal of DDT coverage from fruit trees. The water from under tree sprinklers usually does not wet the fruit on the lower limbs until late in the season when the weight of the fruit bends it into the sprinkler's stream. For this reason, the effect of under-tree sprinklers was not a problem to be investigated at this time. The following work with certain stickers and spreaders and overhead sprinklers was undertaken at the Tree Fruit Experiment Station in Wenatchee, Wash., during the 1947 growing season.

Four adjoining Starking Delicious apple trees, approximately 12 years of age, were selected in order that one overhead sprinkler set up in the center could adequately cover all the trees. An oscillating sprinkler was used with a one-eighth-inch nozzle at 7° above the horizontal. The sprinkler was operated at 22 pounds' pressure, and delivered approximately 5 acre-inches of water during the 24-hour period.

Samples of fruit were taken at random over the entire tree before sprinkling and after 24 hours of continuous sprinkling. The trees were allowed to dry after sprinkling, before the samples were taken. DDT residue was determined by the method given in part I of this paper. Four different spray programs were used. All sprays were applied by one man using a 100-gallon Hardie portable sprayer with 425 pounds' pressure at the pump. The results are shown in table 3.

TABLE 3.—*DDT deposits before and after irrigation by sprinkling for 24 hours*

Plot No.	Spray program [1]	Mean DDT deposit—		Mean difference
		Before sprinkling	After sprinkling	
		P. p. m.	*P. p. m.*	*P. p. m.*
1	2 lbs. 50-percent DDT per 100 gals. of water	13.9	13.1	0.8
2	2 lbs. 50-percent DDT plus 2 qts. light-grade summer petroleum oil per 100 gals. of water	18.8	19.9	−1.1
3	2 lbs. 50-percent DDT plus ⅙ lb. dry-casein-type spreader per 100 gals. of water	15.9	14.0	1.9
4	2 lbs. 50-percent DDT plus ¼ pt. liquid-soap-type spreader per 100 gals. of water	9.1	10.1	−1.0

[1] There were 8 replicates of each treatment.

The addition of 2 quarts of light-grade summer petroleum oil to the spray program (plot 2) gave a significant increase in DDT residue over the spray program on plot 1. The addition of one-sixth pound of dry-casein-type spreader (plot 3) did not give a significant increase or decrease in DDT residue over the spray program on plot 1. The addition of one-fourth pint of liquid-soap-type spreader (plot 4) gave a significant decrease in DDT residue over the spray program on plot 1.

The study of the effect of overhead irrigation sprinklers on the removal of DDT indicated that 24 hours of overhead sprinkling did not reduce the amount of DDT adhering to the fruit. In no case was there a significant difference in DDT residue before and after sprinkling.

SUMMARY

The results of two studies relating to DDT spray residue on apples are reported herein. The first relates to the effect of washing on the removal of DDT residue, the second to the effect of certain stickers and spreaders on DDT residue and of overhead sprinkling on removal of DDT residue.

The results of the first study may be summarized as follows:

When 42 apple samples with an original DDT residue ranging from 5.0 to 22.3 parts per million were washed by three different treatments, a high percentage of the washed samples had a significant amount of DDT residue removed. Nevertheless, none of the washing treatments removed sufficient residue from all samples to make these treatments practicable for commercial use. Sixty pounds of sodium silicate per 100 gallons of water at 100° F., in a single-unit washer gave a more efficient DDT residue removal than 1.5 percent hydrochloric acid at 100° F., in a single-unit washer, and was equally as effective as the tandem wash of sodium silicate followed by hydrochloric acid.

When the original DDT residue on apples exceeded 10 parts per million, the standard washing practices in north-central Washington were not sufficient to consistently reduce this residue to a point equal to or less than the permissible tolerance limit of 7 parts per million.

In the second study it was found that—

(1) The addition of light-grade petroleum oil significantly increased the DDT residue over the straight DDT spray.

(2) The addition of dry-casein-type spreader did not significantly increase the DDT residue over the straight DDT spray.

(3) The addition of liquid-soap-type spreader significantly decreased the DDT residue over the straight DDT spray.

(4) Twenty-four hours of overhead sprinkling irrigation with an oscillating sprinkler that delivers approximately 5 acre-inches of water in a 24-hour period did not remove significant amounts of DDT residue from fruit sprayed with wettable DDT, wettable DDT and petroleum oil, wettable DDT and dry-casein-type spreader, or DDT and liquid-soap-type spreader.

COMPOSITION AND DIGESTIBLE ENERGY OF HAYS FED TO CATTLE[1]

By T. G. PHILLIPS, *chemist*, and M. E. LOUGHLIN, *assistant chemist, New Hampshire Agricultural Experiment Station*

INTRODUCTION

The evaluation of feeds is the subject of a report by a committee of the American Society of Animal Production written by Mitchell (*10*).[2] This reviewed the relations that have been found to exist between constituents determined chemically and certain items in the utilization of feeds. The close negative relation between crude fiber content and the digestibility of the organic matter of feeds by different species of animals was emphasized especially. The committee suggested that further studies along these lines might yield valuable results.

Dissatisfaction with the standard methods of analysis has been expressed frequently. Crampton and Forshaw (*4*) among others, have attempted to use more detailed determinations, especially of the carbohydrate fractions, in studies of the relation of composition to the usefulness of feeds. The results have been disappointing. So far no carbohydrates or grouping of carbohydrates known or supposed to be utilizable have been found which are clearly related to the energy animals can obtain from feeds.

In the present limited study it was hoped that some definite relations between composition and useful energy might be found if the number of variables were reduced as far as possible. For this purpose investigators known to have conducted experiments on the energy metabolism of farm animals were asked if they could supply samples of essentially pure stands of grasses or hays that had been used as the sole ration in energy utilization studies with cattle. To supplement the data supplied by the cooperating investigators, these samples were to be analyzed in this laboratory as completely as practicable, with especial attention to the carbohydrate fractions. The items of composition were then to be compared with the results of the digestion experiments.

MATERIAL AND METHODS

The conditions proposed above limited the number of samples that could be found. Eventually 25 were received, as follows: 10 of

[1] Received for publication June 8, 1948. Scientific contribution No. 120 of the New Hampshire Agricultural Experiment Station.

[2] Italic numbers in parentheses refer to Literature Cited, p. 395.

timothy, of which No. 9 was grass fed fresh; 1 of bromegrass; 1 of oat hay; 1 of Sudan grass; 1 of mixed grass, largely redtop, fed fresh (No. 8); 7 of alfalfa; 1 of red clover; 1 of alsike; 1 of soybean hay; 1 of lespedeza. With the 2 exceptions noted, all were fed as hay.[3]

Accompanying each sample were the results that had been obtained for digestible energy and the digestibility of the dry matter. In many cases the metabolizable energy had been determined either directly or by calculation. The results of the standard feeding-stuff analyses also were provided with many of the samples.

The samples were analyzed in the writers' laboratory by the methods used in a previous study of timothy (*11*) except that the extraction was modified for dry samples. These were extracted in a Soxhlet apparatus, first with anhydrous ethyl ether, then with 95 percent ethyl alcohol. The material extracted by alcohol did not all remain in solution but dissolved when enough water was added to make the alcohol concentration 80 percent.

The items or groups used in the comparisons were (1) protein, (2) lignin, (3) cellulose, (4) hemicellulose, (5) total carbohydrate as suggested by Crampton and Whiting (*6*) including hemicellulose, starch if present, fructosans if present, and the sugars, all calculated to the starch equivalent, and (6) crude fiber.

These categories of materials were compared with (1) the digestibility of the energy, (2) the digestible energy in calories per gram, and (3) the digestibility of the dry matter. Since the heats of combustion of the samples differed only slightly the relations of constituents to (1) and (2) are so nearly identical that only the former are presented. The relations to (1) and (3) also are quite similar. These relationships were graphed and the corresponding correlation coefficients and regression equations were calculated.

RESULTS OF ANALYSES

It was found that metabolizable energy had not been determined for 7 of the samples and that the data for calculating it were not available. Therefore this item could not be used in the comparisons. The relation between metabolizable energy and digestible energy for the remaining 18 samples was plotted and the correlation coefficient was calculated. The graph was a straight line one with $r=+0.948$. In this limited group of samples, then, the relation between these items is very close.

The data used in the rest of the calculations are presented in table 1. The figures for digestibility and many of those for crude fiber were supplied by the cooperating investigators. The remainder of the items were determined in this laboratory.

[3] The cooperation of the following, who supplied one or more of the samples and the relevant data, is gratefully acknowledged: J. A. Newlander, University of Vermont; Max Kleiber, University of California; H. H. Mitchell, University of Illinois; H. A. Herman, University of Missouri; E. G. Ritzman, University of New Hampshire; R. W. Swift, Pennsylvania State College.

TABLE 1.—*Digestibility and composition of hays*

Sample No.	Species	Digestible energy	Digestibility of—		Composition (percent of dry weight) of—					
			Energy	Dry matter	Protein	Hemicellulose	Total carbohydrates	Cellulose	Lignin	Crude fiber
		Cal. per gm.	*Percent*	*Percent*						
7	Timothy	2,567	57.7	60.4	5.98	14.83	28.95	30.14	9.85	33.0
9	do	3,534	78.6	80.8	11.36	13.28	25.51	24.94	6.92	24.0
12	do	2,493	55.7	56.7	5.41	17.10	27.28	33.72	10.56	34.8
15	do	2,498	56.4	59.3	8.31	15.93	19.91	32.62	10.51	33.5
16	do	2,856	62.4	64.4	8.00	17.61	22.01	37.20	9.60	34.7
17	do	2,387	53.2	54.8	5.89	15.57	25.98	34.53	11.49	34.1
19	do	2,232	49.6	47.3	7.27	16.34	22.81	34.20	11.53	34.9
22	do	2,785	62.0	65.3	9.07	17.20	23.39	30.54	9.69	33.1
24	do	2,569	59.6	61.7	9.44	13.51	23.82	28.13	9.82	30.0
25	do	2,828	64.6	67.2	8.78	16.07	23.77	27.12	8.16	29.0
1	Bromegrass	2,949	67.6	69.4	12.15	15.97	20.85	30.98	7.58	31.1
6	Oat hay	3,033	66.8	67.5	13.75	15.61	18.95	30.52	8.54	30.0
8	Mixed	2,332	51.7	62.5	7.28	14.89	25.54	31.29	9.45	32.8
23	Sudan	2,861	67.8		12.59	13.62	22.22	27.90	8.24	27.2
2	Alfalfa	2,670	59.7	60.4	16.73	8.84	13.90	27.09	11.14	33.4
3	do	3,032	67.0	67.6	22.00	8.26	13.52	23.51	8.97	26.1
10	do	2,809	61.2	62.2	18.47	9.60	11.80	25.50	10.74	30.9
11	do	2,463	54.5	56.2	14.43	9.76	12.35	32.60	11.92	32.8
14	do	2,508	56.7	59.0	16.26	10.63	12.48	28.66	11.12	32.8
18	do	2,659	58.1	59.2	17.84	9.34	11.65	26.88	11.01	30.6
21	do	2,466	54.7	56.6	13.78	9.52	13.08	34.00	12.91	40.5
4	Red clover	2,599	60.1	59.9	12.50	8.75	16.01	28.77	11.00	30.4
5	Alsike	2,522	57.0	58.8	11.24	12.58	15.80	31.61	11.43	30.2
20	Soybean hay	2,889	63.7	60.7	11.24	13.15	19.54	35.20	11.38	33.1
26	Lespedeza	2,596	57.5	58.0	12.15	9.29	16.01	27.19	13.19	32.2

The relation between the protein content of the samples of timothy and alfalfa and the digestibility of their energy is shown in figure 1.

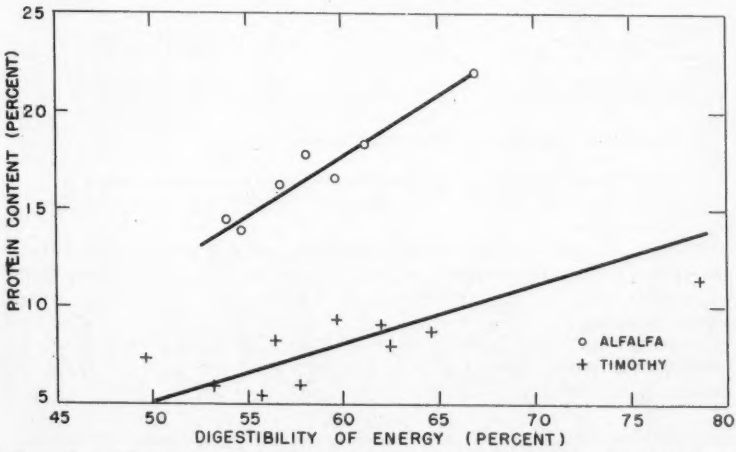

FIGURE 1.—Relation between protein content of the samples of timothy and alfalfa and the digestibility of their energy. For alfalfa $r=+0.966$, for timothy $r=+0.786$, for both $r=+0.244$.

In each of the species this relation is highly significant, but at different levels of protein content. When all 17 samples are considered the relation is not significant. Similar specific differences were evident to a greater or less extent in all the relations studied.

It was considered advisable then to study the 10 timothy samples and the 7 alfalfa samples separately and to ignore the other species. This reduced the number of samples in each comparison greatly and so decreased the value of the results.

Such relations among the major items considered as were found to be significant are listed in table 2. No significant relations were found between digestibility and content of hemicellulose or of total carbohydrates.

For the timothy and alfalfa samples, separately, the regression equations for calculating the digestibility of the energy and the digestibility of the dry matter, respectively, from the content of lignin, of crude fiber, and of protein are presented in table 3. Also included are the direct correlation coefficients, the standard deviations of the two digestibilities, and the standard error of estimate of each of these when calculated from the constituent indicated.

The large "a" values in some of these equations show the danger of extrapolating beyond the range of the data at hand. They indicate either that the relations would not follow a straight line at extreme values of the variables, or that a wider range of data would result in an appreciable change in the slope of the regression line.

TABLE 2.—*Correlation coefficients*

Variables	Percent lignin	Percent crude fiber	Percent protein	Percent cellulose
Timothy:				
Digestibility of energy	−0.962	−0.872	+0.786	−0.689*
Digestibility of dry matter	−.954	−.842	+.761*	−.689*
Alfalfa:				
Digestibility of energy	−.932	−.787*	+.966	−.899
Digestibility of dry matter	−.939	−.784*	+.966	−.889

*Significant at the 5-percent level; all others at the 1-percent level.

TABLE 3.—*Regression equations, digestibility of constituents*

TIMOTHY

y	*x*	*r-xy*	Equation	*Sy*	*Sy. x*
	Percent				
Digestibility of energy	Lignin	−0.962	$y=113.1-5.41x$	7.94	2.29
	Crude fiber	−.872	$y=123.8-2.00x$	7.94	4.13
	Protein	+.786	$y=33.2+3.37x$	7.94	5.21
Digestibility of dry matter	Lignin	−.954	$y=120.3-5.96x$	8.84	2.83
	Crude fiber	−.842	$y=130.3-2.14x$	8.84	5.06
	Protein	+.761	$y=33.0+3.62x$	8.84	6.08

ALFALFA

Digestibility of energy	Lignin	−0.932	$y=96.5-3.39x$	4.36	1.73
	Crude fiber	−.787	$y=84.6-0.79x$	4.36	2.95
	Protein	+.966	$y=32.7+1.53x$	4.36	1.24
Digestibility of dry matter	Lignin	−.939	$y=93.9-3.04x$	3.88	1.46
	Crude fiber	−.784	$y=83.0-0.70x$	3.88	2.64
	Protein	+.966	$y=36.9+1.36x$	3.88	1.10

DISCUSSION

Crampton and Forshaw (*4*) compared the gains in weight of rabbits with the composition of the grass clippings used as feed. Lignin was negatively correlated with gains, but the result did not reach even the 5-percent level of significance. The very high correlation of −0.978 was found by Lancaster (*8*) between the lignin content of 14 feeds and the digestibility of their organic matter by sheep. The feeds included both succulents and hays. Lancaster concluded that the superiority of lignin as a factor in feed evaluation is not clearly established. The determination is not yet satisfactory and further studies are needed.

The results of the present study give further evidence of the importance of this relation. Lignin content is closely related to digestibility of energy in both timothy and alfalfa, and thus in the limited sampling available, it is shown to be an excellent basis for calculating digestibility in each of the species. It should be noted, however, that the regression coefficients, which measure the change in digestibility corresponding to an increase of 1 percent in lignin content, are strikingly different for the two species, −5.41 for timothy and −3.39 for alfalfa (table 3).

Reports on the relation of crude fiber to the digestibility of the organic matter of feeds are more numerous. Some recent results are summarized in table 4. McMeekan's figures include a wide variety

TABLE 4.—*Relation between digestibility of organic matter and crude fiber conten*

Source of data	Number of items	Correlation coefficient	Regression coefficient
Cattle:			
Axelsson (*1*)			−0.879
Brouwer and Dijkstra (*3*)	9	−0.784	−1.134
Brouwer and Dijkstra (*3*)[1]	10	−.557	−.515
Duckworth (*7*) American cattle [1]	101	−.680	−.918
Duckworth (*7*) Zebu cattle [1]	166	−.417	−.511
Sheep:			
Brouwer and Dijkstra (*3*)[1]	38	−.674	−.992
Brouwer and Dijkstra (*3*)[1]	22	−.525	−.720
Brouwer and Dijkstra (*3*)[1]	15	−.599	−.479
Brouwer and Dijkstra (*3*)[1]	48	−.518	−.564
Lancaster (*8*)	14	−.944	[2] −1.698
McMeekan (*9*)[1]	50	−.944	−.960

[1] Calculated from the results of digestion experiments assembled from the literature.
[2] Calculated from Lancaster's data.

of feeds, succulents, silages, roughages, and concentrates. Lancaster's include succulents and roughages. The rest are largely limited to grasses and hays. Duckworth included only roughages exclusive of silages.

All the correlation coefficients listed in table 4 show definite trends, but only the highest ones indicate a relationship close enough to permit the constituent to be used as a reasonably satisfactory measure of digestibility.

The regression coefficients also vary widely. Axelsson (*2*) has explained some of the variations in this figure on the basis of differences in experimental technique and has emphasized the need for the standardization of methods in digestibility trials.

The results reported here for crude fiber (tables 2 and 3) are well within the range of those assembled from the literature (table 4). In

the samplings of both timothy and alfalfa the relation between crude fiber content and digestibility of energy is significant, though not as close as the lignin relationship. Here, again, the differences in the regression coefficients relating differences in crude fiber content to differences in digestibility are striking, −2.00 for timothy and −0.79 for alfalfa.

The regression coefficients found by other workers (table 4) also vary widely, from −0.479 to −1.698. It is quite impossible to select from these varying values one which could be used with confidence in calculating the digestible energy of feeds from their crude fiber content.

In the alfalfa samples the protein content is very closely related to the digestibility of energy, but in the timothy samples this relation barely reaches the 1-percent level of significance.

The cellulose relationships differ in the same way as those of protein but are at an appreciably lower level of significance.

For neither timothy nor alfalfa do any of the groupings of carbohydrates studied show a significant relation to the energy yield. These results confirm at least the latter part of a statement made by Crampton and Jackson (*5, p. 339*), "Protein and fiber fail as indices of nutritive value as do all other fractions reported in this paper."

The relations found in this study are limited in value because of the small number of samples included. Those with lignin, however, are extremely close, and different for the two species. It is likely that they would be much less marked in studies of mixed herbage whose botanical composition varied widely.

SUMMARY

Twenty-five samples of hays received from various laboratories in the United States were studied. These hays had served as the sole ration in feeding experiments with cattle. Each sample was from an essentially pure stand of a single species of grass or legume.

The samples were analyzed chemically and their composition was compared with the results of the feeding trials.

Comparison of the digestible and the metabolizable energy of the 18 samples for which the latter value had been determined show a very close relation between the two.

Species differences were so pronounced that it was considered advisable to make separate studies of the 10 samples of timothy and the 7 of alfalfa. The other samples, one each of 8 species, were not included. Thus the number of samples used in the comparisons was small.

The lignin, protein, cellulose, and crude fiber content of the samples all are related more or less closely to their yield of energy but at differing levels of these constituents for the two species.

When applied to the samples of each of the two species separately, the lignin content serves as an excellent means of estimating the digestibility of energy and of dry matter.

Crude fiber content also is related significantly to the digestibility of energy, but less closely than the lignin content.

The regression coefficients relating differences in a constituent to differences in digestibility differ widely for the two species.

The carbohydrate fractions studied, other than cellulose, show no significant relation to the digestibility of either energy or dry matter.

LITERATURE CITED

(1) Axelsson, J.
1938. DIE BESTIMMUNG DES ALLGEMEINEN NÄHRWERTES (ENERGIEWERTES). DER FUTTERMITTEL NACH DER CHEMISCHEN ZUSAMMENSETZUNG. Biedermanns Zentbl. f. Agr. Chem., Abt. B, Tierernährung 10: 238–248, illus.

(2) ———
1940. BEDEUTUNG UND WERT DER ROHFASER FÜR DAS FUTTER DES RINDES Biedermanns Zentbl. f. Agr. Chem., Abt. B, Tierernährung 12: 414–443, illus.

(3) Brouwer, E., and Dijkstra, N. D.
1938. ONDERZOEK NAAR DE VOEDERWAARDE VAN NEDERLANDSCHE HOOISOORTEN EN NAAR HUN INVLOED OP DE EIWIT—, KALK— EN PHOSPHORSTOFWISSELING BIJ JONGE RUNDEREN. Verslag. Landb. Onderzoek. 44, C: 529–612, illus.

(4) Crampton, E. W., and Forshaw, R.
1940. PASTURE STUDIES XVI. THE NUTRITIVE VALUES OF KENTUCKY BLUE GRASS, RED TOP AND BROME GRASS WITH PARTICULAR REFERENCE TO THE RELATION BETWEEN THE CHEMICAL COMPOSITION OF THE HERBAGES AND THE LIVE WEIGHT GAINS MADE BY THE ANIMALS SUBSISTING THEREON. Jour. Nutr. 19: 161–172.

(5) ——— and Jackson, I. R. C.
1944. SEASONAL VARIATION IN CHEMICAL COMPOSITION OF PASTURE HERBAGE AND THE RELATION TO ITS DIGESTIBILITY BY STEERS AND SHEEP. (PASTURE STUDIES—XXVI.)Jour. Anim. Sci. 3: [333]–339, illus.

(6) ——— and Whiting, F.
1943. A PROPOSED SCHEME OF FEEDINGSTUFFS ANALYSIS. Jour. Anim. Sci. 2: [278], illus.

(7) Duckworth, J.
1946. A STATISTICAL COMPARISON OF THE INFLUENCE OF CRUDE FIBRE ON THE DIGESTIBILITY OF ROUGHAGE BY BOS INDICUS (ZEBU) AND BOS TAURUS CATTLE. Trop. Agr. [Trinidad] 23: 4–8, illus.

(8) Lancaster, R. J.
1943. METABOLISM TRIALS WITH NEW ZEALAND FEEDING-STUFFS IV. THE RELATIVE SIGNIFICANCE OF LIGNIN, CELLULOSE, AND CRUDE FIBRE IN THE EVALUATION OF FOODS. New Zeal. Jour. Sci. and Technol. 25A: 137–151.

(9) McMeekan, C. P.
1943. A NOTE ON THE RELATIONSHIP BETWEEN CRUDE FIBRE AND THE DIGESTIBILITY OF ORGANIC MATTER. New Zeal. Jour. Sci. and Technol. 25A: 152–153.

(10) Mitchell, H. H.
1942. THE EVALUATION OF FEEDS ON THE BASIS OF DIGESTIBLE AND METABOLIZABLE NUTRIENTS. Jour. Anim. Sci. 1: [159]–173.

(11) Phillips, T. G., and Smith, T. O.
1943. THE COMPOSITION OF TIMOTHY. I. YOUNG GRASS AND HAY. N. H. Agr. Expt. Sta. Tech. Bul. 81: [3–16.]

U. S. GOVERNMENT PRINTING OFFICE: 1949